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CONTENTS

PAGE

A Study of the Clotting of the Plasma of Frog's Blood and the Transformation of the Clot into a Fibrous Tissue. *George A. Baitsell*..... 109

The Volume of the Blood During Hibernation and Other Periods of the Year in the Woodchuck (Marmota Monax). *Andrew T. Rasmussen and Gertrude B. Rasmussen*..... 132

The Relation of the Rate of the Spontaneous Liberation of Epinephrin to the Rate of Blood Flow Through the Adrenals. *G. N. Stewart and J. M. Rogoff*.. 149

The Effect of Varying the Calcium and Potassium Content of Perfusion Fluids on Vagus Inhibition. *Blanche M. Brine*..... 171

The Action of Adrenalin on the Blood. *M. L. Menten*..... 176

The Effect of the Accumulation of Carbon Dioxide on the Tidal Air and on the H-Ion Concentration of the Arterial Blood in the Decerebrate Cat. *R. W. Scott*..... 196

The Skin of the Catfish (Amiurus Nebulosus) as a Receptive Organ for Light. *Anne P. Van Heusen*..... 212

The Effects of Adrenin on the Distribution of the Blood. VI. Venous Discharge from the Thyroid Glands. *R. E. Lee Gunning*..... 215

Contributions to the Physiology of the Stomach. XLII. Hunger and Appetite in Fever. *Jacob Meyer and A. J. Carlson*..... 222

Contributions to the Physiology of the Stomach. XLIII. The Influence of Experimental Ulcers of the Stomach and Duodenum on the Hunger Contractions of the Empty Stomach. *J. R. Dundon*..... 234

On the Genesis and Inhibition of Extensor Rigidity. *Stanley Cobb, Albert A. Bailey and Paul R. Holtz*..... 239

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AUTHOR'S CORRECTION

(Insert opposite page 444, Volume xliii, 1917)

The sentence (page 444, paragraph 2) beginning: "It should be noted, however," should be interchanged with the first part of a sentence on page 450 (paragraph 1), viz., with "It should be noted that . . . . the positions were posterior."

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A STUDY OF THE CLOTTING OF THE PLASMA OF FROG'S BLOOD AND THE TRANSFORMATION OF THE CLOT INTO A FIBROUS TISSUE

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From the Osborn Zoölogical Laboratory, Yale University

Received for publication June 25, 1917

INTRODUCTION

In two previous papers the author has shown that a fibrin clot formed from frog plasma may be transformed into a fibrous tissue which resembles connective tissue. In the first paper[1] the transformation of the fibrin clot was observed and studied in tissue cultures and in brief it was shown that in a number of living cultures of various frog tissues changes in the fibrin net began when the culture was from two to three days old, and as a result, a great number of fine wavy fibrils were formed. These united to form wavy bundles of fibers which freely intertwined and anastomosed as they ramified through the area of the plasma clot. In a number of instances, in the course of a few days, the plasma clot surrounding the imbedded living tissue became almost completely transformed by a fusion and consolidation of the fibrin elements, into the new fibrous tissue.

In the second paper[2] the action of the fibrin clot in wound healing was studied, This work for the most part was carried on in the skin of adult frogs and it was shown that, at first, in the experimental

[1] Baitsell: The origin and structure of a fibrous tissue which appears in living cultures of adult frog tissues. Journ. Exper. Med., 1915, xxi, 455.

[2] Baitsell: The origin and structure of a fibrous tissue formed in wound healing. Journ.Exper. Med., 1916, xxiii, 739.

wounds there was a coagulation tissue formed by the clotting of the blood and lymph and in this a typical fibrin net was present. Later this fibrin net was directly transformed into a new fibrous tissue containing bundles of wavy fibers as noted above. In fact this process was found to be identical with that which had been previously found to take place in the fibrin clots in living cultures of adult frog tissues.

Having shown by the above mentioned observations that the fibrin net of a clot of frog plasma may be directly transformed into a fibrous tissue and also that this phenomenon regularly occurs and is, apparently, an important factor in wound healing in the frog, it appeared necessary, for a complete understanding of the reaction, to study preparations of normal fresh frog plasma, observe the details of clotting and, if possible, the formation of the fibrous tissue. It was hoped that the use of living tissues in connection with the transformation of the clot might be dispensed with and the formation of the fibrous tissue controlled by mechanical factors applied directly to the clot. In the study of the transformation of the clot in tissue cultures and also in wound healing, living tissues were present and although it was clear that the formation of the fibers was not due to an intracellular action, all possible questions as to their origin would be avoided by the complete elimination of the living tissues from the plasma. The results of these experiments, which are recorded in the present paper,[3] show that such a procedure is possible. It has been found that a plasma clot may be subjected to various mechancial factors and, as a result, it will lose its typical normal structure and become transformed into a new fibrous tissue. In other words, normal clotted blood plasma of the frog, by the introduction of a mechanical factor, may be transformed, through a fusion of the elements of the fibrin net, into a fibrous tissue which in its histological structure is apparently identical with various types of normal connective tissues of the frog.

In brief then, the results of the present experiments which are reported in this paper enable us to complete the story of the new fibrous tissue which was started in the two previous papers, to trace its complete history and control its formation from the time the plasma is taken from the animal until the new fibrous tissue is formed by a direct transformation of the clot under the influence of various mechanical factors.

[3] Abstracts of portions of this paper appear in the Proc. of the Soc. for Exper. Biol. and Med., 1916, xiii, 194, and also in the Proc. of the American Physiological Society published in this Journal, 1917, xlii, 608.

OBSERVATIONS ON THE CLOTTING OF FROG PLASMA

Results of previous workers. The investigations of previous workers on the clotting of blood plasma of various animals have given rise to two views as to the method by which a clot is formed. Mayer[4] and Cesana,[5] both working with the ultramicroscope, state as a result of their observations that clotting occurs by a process of agglutination of minute particles to form threads or masses of fibrin. Even after these are formed it is possible to distinguish the individual particles of which they are composed.

Stübel[6] working with a dark field apparatus and Howell[7] with the ultramicroscope have both concluded from their observations that the clotting of blood plasma resembles a process of crystallization in which the fibrin is deposited as definite, well-formed needles or rods. This method of clot formation holds true according to both of these authors for the normal plasma of all the vertebrate animals tried with the exception of the plasma of birds and frogs.

Howell[8] in a more recent paper confirms and emphasizes his previous results as to the method of normal clot formation of fresh plasma from various animals. This normal type of clot he terms a "crystalline gel," in distinction to a second type of clot which may form in old plasma or in plasma which has been treated in various ways which, in general, result in a decrease in the alkalinity. This type of clot he terms a "structureless gel" owing to the fact that during its formation the fibrin elements which form are so minute that they are beyond the resolving power of the ultramicroscope and the clot appears as a structureless mass.

Methods used in the present experiments. In the present study, the phenomena associated with the formation of the plasma clot from frog's blood have been observed by means of dark field illumination. The Leitz apparatus was used. It consisted of a dark field condenser

[4] Mayer: La coagulation du plasma sanguin. Compt. rend. de la Soc. de Biol., 1907, lxiii, 658.

[5] Cesana: Ricerche ultramicroscopiche sulla coagulazione del plasma di sangue. Arch. di Fisiol., 1908, v, 180.

[6] Stübel: Ultramikroskopische Studien über Blutgerinnung und Thrombocyten. Pflüger's Arch., 1914, clvi, 361.

[7] Howell: The clotting of blood as seen with the ultramicroscope. This Journal, 1914, xxxv, 143.

[8] Howell: Structure of the fibrin-gel and theories of gel-formation. This Journal, 1916, xl, 526.

which was fitted in the microscope in place of the regular condenser and a 5 ampere arc light, with rheostat, for illumination. A $\frac{1}{12}$ oil immersion lens with either a number 4 or 8 compensating ocular was used. With this equipment a magnification of 700 diameters or 1340 diameters could be obtained which was sufficient to observe the details of the reaction without great difficulty.

The technique employed in obtaining the plasma of the frog was the same as previously used in the tissue culture work and has been described in a previous paper. In this method an etherized frog is fastened with the ventral surface up to a frog board. The heart is then exposed and a few cubic centimeters of blood drawn by means of a Luer 5 cc. glass syringe, the needle of which is inserted into the ventricle of the heart. The blood which is thus obtained is put into a sterile paraffine-covered tube and centrifuged for about four minutes. The clear plasma is then drawn off with a pipet and placed in small sterile paraffine-covered test tubes. The plasma may be used at once or placed in the refrigerator until needed.

For observation of the phenomena associated with clotting, a few drops of the plasma were placed on a regular sized glass slide and covered with a 22 mm. square cover glass. Sufficient plasma was used to prevent the cover glass coming into too close contact with the slide. The slide was at once placed on the microscope and the observations made.

The clotting of frog plasma as observed with dark field illumination. The fresh plasma of the frog in most cases begins to coagulate almost as soon as it is placed on the slide. When first observed the field is quite dark and only a few small round particles which are made luminous by the indirect illumination are to be seen. These particles show a Brownian movement. An observation of the same field a few minutes later will show that these particles have greatly increased in number and also in the rapidity of their movements and the field under observation will present quite an animated appearance. These vibrating particles in the frog plasma do not appear to have any direct connection with the fibrin elements of the clot. There is a considerable variation in the time of clotting depending upon the condition of the plasma, temperature of the room and various other factors but, in general, the first of the fibrin elements will be seen within a few minutes after the plasma is exposed. These are first seen as fine distinct rods or filaments and they are somewhat smaller in diameter than the round particles which appear first. There are only a few of the fibrin elements at first and these float through the plasma and rapidly in-

crease in length. They are not rigid structures but, on the contrary, when they have reached a considerable length they bend back and forth in response to currents which are set up in the plasma. The formation of these fibrin filaments and the round particles as well apparently occurs through a fusion of particles in the plasma which are too small to be seen with the equipment used in these experiments. It is possible to focus on the end of a growing filament and to note, from time to time, the increase in length. A few minutes after the first fibrin filaments are observed, it will be found that they are present in such large numbers that they are no longer able to float through the field in the uncoagulated plasma without coming in contact with each other. The observations show that the fibrin filaments fuse very readily with each other. In some cases it is clear that filaments which float together in the uncoagulated plasma remain attached and such a condition will give rise to branched filaments. It also frequently happens that a number of filaments may become intermeshed to form a small piece of network which may float through the plasma which still remains uncoagulated.

When the phenomena associated with the clotting have reached the maximum the field under observation presents a very animated appearance. Both the round vibrating particles and the fibrin elements have greatly increased in number and in the vigor of their movements and as a result the field, which was quite dark when first observed a few minutes before, has become quite luminous. Some of the plasma is still uncoagulated at this stage and great numbers of the fibrin filaments, some single, others intermeshed and cohering to form various sized pieces of the fibrin net, float through the field. A few minutes later the clot will be completely formed and the field under observation then shows a meshwork made up of the hairlike fibrin filaments. Lying on the filaments and also scattered among the meshes of the network great numbers of the small, round particles are to be seen.

It may be well at this stage to note some of the figures showing the phenomena associated with clotting. The figures shown are photomicrographs at 334 diameters which were taken from the fresh preparations, with dark field illumination, at various stages of the process of clotting.

Figure 1 shows an early stage in the process of clot formation. The field is quite dark. Numbers of the round vibrating particles are to be seen. These vary considerably in size. The fibrin elements are

forming as separate, distinct filaments which rapidly lengthen and bend back and forth with the flow of the plasma. This movement can be augmented by gently touching the cover glass of the preparation with the microscope objective. Under such conditions it can be seen that the fibrin filaments are very flexible. In the center of the figure it will be noted that the filaments are becoming intermeshed to form a network.

Figure 2 shows a more advanced stage of clot formation. The field has become considerably lighter owing to the great increase in the number of the round, vibrating particles and in the numbers of fibrin filaments, both of which add considerably to the luminosity of the field. The filaments have intermingled and cohered to form a quite definite net in various regions of the preparation. Separate fibrin filaments are also to be observed.

Figure 3 shows the clot practically formed. The entire field is covered with a fine network which has resulted from the coherence and intermingling of the fibrin filaments. Very little of the liquid plasma remains at this stage and with its coagulation the Brownian movement of the round particles has very largely ceased. Some are shown in the figure but great numbers of them have come to rest in the interstices of the network and on the fibrin filaments.

Fig. 1. Fresh preparation of blood plasma of the frog. × 334. Taken with dark field illumination. The preparation shows an early stage in the process of clot formation. Numerous distinct fibrin elements are to be seen and also a considerable number of the small round particles which show a vigorous Brownian movement during clot formation. In the center of the figure the fibrin elements are becoming intermeshed to form a network.

Fig. 2. Same as in figure 1. This figure shows a later stage in clot formation. The field is much lighter in color which is largely due to the increased amount of fibrin material present.

Fig. 3. Same as in figure 1. This figure shows the process of clot formation in the plasma almost completed. The field is covered with a fine network which has been formed by the deposition of the distinct fibrin elements. (Cf. figure 9 which shows a portion of a normal clot after it has been preserved and stained.)

Fig. 4. Same as in figure 1. This figure shows the formation of long nearly parallel fibers by the agglutination of the small round particles. A structure of this kind is in agreement with Mayer's view of clot formation. In frog plasma a formation of this kind apparently occurs only in that part of the plasma which is in contact with the cover glass or slide. It is believed that in the frog such a structure does not represent the normal method of fibrin formation and it may not be connected with the normal process as shown in figures 1 to 3. The light colored area to the right of the figure is due to reflection.

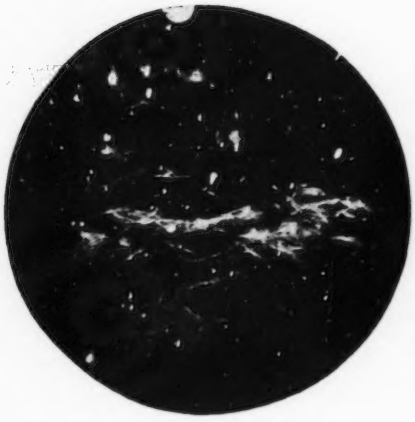

Fig. 1

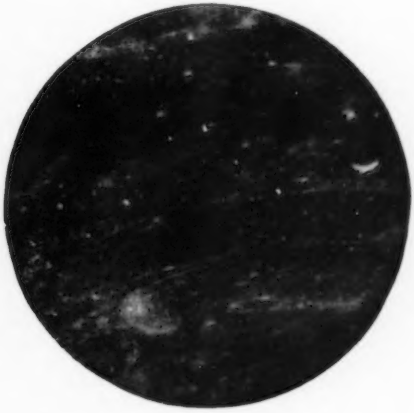

Fig. 2

Fig. 3

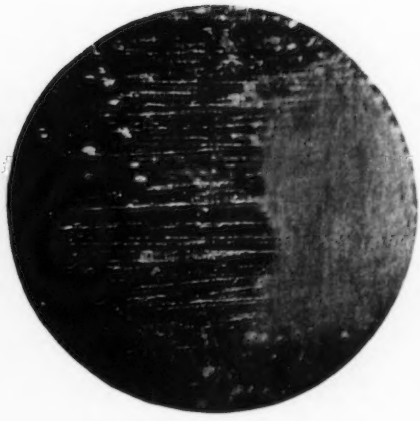

Fig. 4

In figures 1 to 3 the formation of a normal clot of frog plasma has been shown. A type of formation such as this corresponds in general to what Howell has termed the "crystalline gel." Figure 4 shows a different condition. In this case it will be noted that long nearly parallel filaments are present. These have arisen, not by the formation of distinct fibrin filaments, but by an agglutination of the round particles. Each of the parallel filaments present in this figure will be found by a careful examination to be composed of particles which have arranged themselves in rows which for the most part are almost parallel. A clot formation of this type is in accordance with the description of clot formation as given by Mayer.[9] Careful observations have convinced me, however, that this method is not the one that normally occurs in frog plasma and, in fact, it may be possible that a reaction such as shown in this figure has no part in the regular fibrin formation. These observations show that a condition such as is present in figure 4 often occurs in the preparations in that portion of the plasma which is in contact with the glass slide or cover slip and that such a condition is apparently due to an adhesion of the vibrating particles to the glass. This adhesion appears to be along the lines of minute scratches in the glass and there is the apparent possibility that it may be chemical in character.

The description given above of the clotting of frog plasma by the formation of separate filaments which intermesh and cohere to form a network is the type of clot which, so far as shown by careful observation of the present experiments, always occurs in fresh, normal frog plasma. However, it has also been definitely observed that the plasma taken from an animal changes with age in respect to the type of clot that it will form. When first observed some hours after having been taken from the animal, the typical "crystalline gel" will form, but if a portion of the same plasma is placed in a refrigerator for a few days and then allowed to clot while under dark field observation, the process of clot formation will be seen to be different. In this case it will be found that no visible fibrin elements are to be observed under the dark field during the clot formation. We have in such a case what Howell has termed a "structureless gel." The formation of this type of clot from old frog plasma does not present nearly as striking a figure under the dark field as in the normal type of clot formation from fresh plasma when a "crystalline gel" is formed. Only comparatively few

[9] Mayer: Op. cit.

of the round, vibrating particles are present and the Brownian movement which they exhibit is not nearly so vigorous. Also there is a complete absence of the definite, visible fibrin elements. The plasma when first observed under the dark field is very dark but in a few minutes gets lighter and as the clotting proceeds takes on a grayish color due presumably to the innumerable, minute fibrin elements which have formed in the plasma and which are too small to be observed individually. When the clot has fully formed the entire field shows a homogeneous gray color.

Transformation of a normal clot. If the fresh plasma has been allowed to clot without disturbing it during the process, the result is the formation of a network made up of distinct fibrin elements as shown in figure 3. However, if the clot has been disturbed during its formation or after it has formed by the action of a mechanical factor such as tension or pressure, the dark field observations show that a striking transformation of the fibrin net results. This pressure can be applied to the coverglass of the preparation while the clot is under observation. The result is a fusion and consolidation of the separate filamentous fibrin elements of the clot to form long fibrillae which group themselves into wavy, fibrous bundles. Additional pressure will cause a still closer union of the fibrillae and a mass of fibrin will be formed which, under the dark field, appears to be more or less homogeneous. This transformation is clearly shown in figures 5 to 8.

Figure 5 shows a rather early stage in the clot formation in which a definite net of the fibrin filaments is forming over most of the field. Pressure was applied for a short time directly to the cover glass of this preparation and the result is shown in figure 6. The fibrin elements of a portion of the net in the center of the field have become more or less consolidated and fused to form what appears as a fibrous bundle made up of long wavy fibrillae. Figure 7 also shows this same transformation of the clot. As a result of tension on the clot the fibrin elements have formed long wavy fibrillae and in the portion of the figure to the right these have become fused into a more or less homogeneous fibrin mass into which their identity is merged. In some cases a considerable portion of the preparation forms a mass of fibrin in which, as far as can be told by dark field observation, a complete fusion of the fibrin elements has occurred and an apparently homogeneous mass of fibrin has resulted from the fusion.

The result of pressure applied to the clot, then, is a fusion and consolidation of the fibrin elements so that from a typical fibrin net made

up of separate intermeshed fibrin elements, as shown in figure 3, a structure may be obtained as shown in figure 8 in which heavy fibrous bands of fibrin have formed. These are of different sizes : nd they intertwine and fuse with each other to a greater or less degree in the different regions of the field, depending apparently upon the direction and amount of pressure and tension exerted.

Transformation of the plasma clot as observed with direct illumination. A normal, undisturbed clot, although the fibrin net is made up of the definite and distinct fibrin elements, is practically transparent and on this account, when studied with a microscope with direct illumination, it is very difficult to make out any structure in the clot whatever. Such a preparation with the direct illumination will show in general only a clear, transparent, homogeneous field. However, after tension has been applied to such a preparation, it is possible under favorable circumstances to see, with direct illumination, wavy fibrous bundles traversing various parts of the clot. To observe their formation it is only necessary to carefully remove the coverglass from the preparation of plasma in which the formation of the clot has been observed under the dark field. The thin film of clot which was formed under observation will generally remain attached to the slide and it can be manipulated there. If such a clot is subjected to tension by means of dissecting needles it can be clearly seen with the direct illumination that long fiber-like structures appear between the two needles along the lines through which the tension is being exerted. These fibers, if the tension is continued, will increase both in length and diameter and

Figs. 5 and 6. As in figure 1. These two figures are from the same preparation. Figure 5 shows a rather advanced stage in the clot formation in which distinct fibrin elements are to be seen. Also, in some regions, their coherence to form a network is shown. Figure 6 shows the same preparation after pressure has been applied to the cover glass. The pressure has resulted in a fusion and consolidation of the fibrin elements of the net in a portion of the field to form long wavy fibrillae.

Fig. 7. As in figure 1. This figure shows the long wavy fibrillae which have resulted from a transformation of the fibrin net under the influence of pressure. To the right of the figure these fibrillae have become closely consolidated to form a more or less homogeneous mass of fibrin. (Cf. figure 10 which shows a portion of a permanent preparation in which a partial transformation of the plasma clot has occurred.)

Fig. 8. As in figure 1. This figure shows a preparation in which the typical fibrin net has, as a result of pressure, become almost completely transformed into a fibrous tissue containing bands of fibers which intertwine and anastomose.

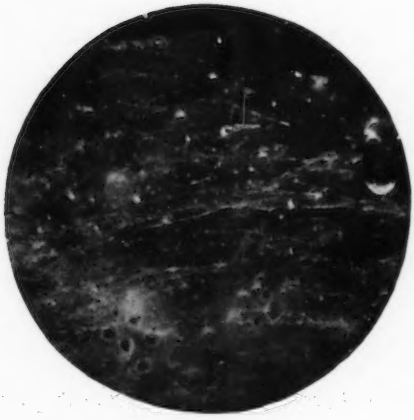

Fig. 5

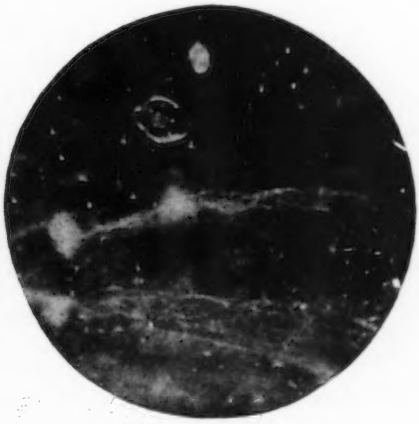

Fig. 6

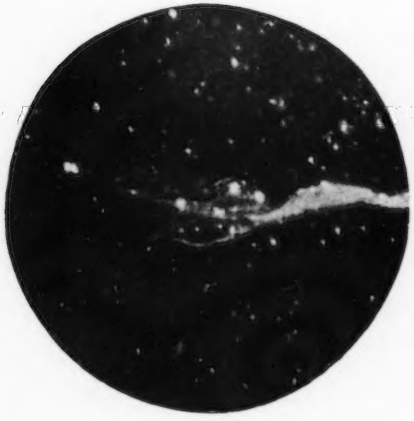

Fig. 7

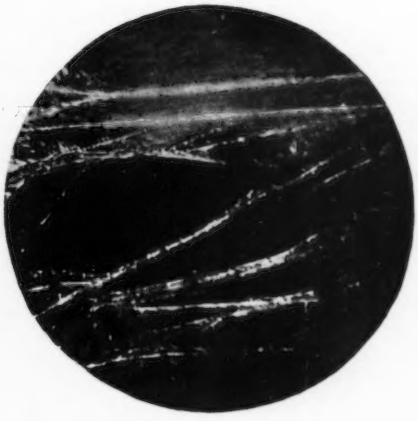

Fig. 8

run across the entire clot. By changing the position of the needles and thus exerting pressure in various directions the fibers can be made to form and to traverse the clot in various directions corresponding to the lines of force exerted by the needles. It is possible in this way to transform a clot which appears to be structureless under the direct illumination to a substance which contains a great number of fibers radiating out in all directions from the points where tension was applied.

This change in the structure of the plasma clot can also be brought about in the following way with the same final result. A few drops of plasma are placed on a slide and allowed to clot. The point of an unpolished dissecting needle is then placed in the clot and slowly revolved. As a result it will be noted that fibers form in the clot at the point where the needle is being revolved. As the process is continued these enlarge and new ones are also formed, so that very shortly the clot contains a great many fibers which radiate out in all directions through it from the point of tension at which the needle was inserted and revolved. It appears evident in such cases that some of the fine threadlike filaments which compose the fibrin net of the clot become attached to the needle and as it is revolved they are drawn together and consolidated to form the fibers. Other fibrin elements are in turn involved and in a short time the fibrin elements of the entire clot have become involved, drawn together and fused to form the fibers which radiate from the point of tension.

Various other methods may be used to bring about the transformation of the plasma clot. Mention of some of these will be made later in the description of the permanent preparations which were preserved for microscopic study. It may be stated that with any of these methods it appears that the same principle holds good, namely, that the structure of the plasma clot can be transformed by the action of mechanical factors, such as tension and pressure, as a result of which a fusion and consolidation of the fibrin elements takes place.

These observations on the clotting of frog plasma as given above agree, in general, with those obtained by Stübel and also by Howell in their experiments with the plasma of various animals and show that normally a "crystalline gel" is formed by the deposition of distinct fibrin elements. They also show that a "structureless gel," as termed by Howell, may be formed in old frog plasma. The present results do not agree with those of Stübel[10] with regard to frog plasma. He

[10] Stübel: Op. cit., 391.

says that in the plasma of this animal, in distinction to that of other animals he studied, the fibrin is deposited as a network instead of being deposited as separate elements.

Howell[11] has evidently not observed the fusion of the fibrin elements in his preparations of the plasma of various animals. He says of the fibrin elements that they "stand out brilliantly in the field as rigid acicular structures." As mentioned above, the present observations show that, in frog plasma, the fibrin elements are generally formed as long rods or filaments which are quite flexible and wave back and forth in the currents set up in the uncoagulated plasma.

Retraction of the clot. In the light of the present results it may be well to mention the phenomena of retraction which, as is well known, is of general occurrence and of great importance in clots. Howell[12] shows that retraction does not take place in a clot which forms a "structureless gel" but only in the "crystalline gel" in which type of clot the distinct, loosely intermeshed fibrin elements are present.

It appears clear from the present observations that the retraction of the clot is due to a fusion and consolidation of the distinct fibrin elements of the clot. This is clearly shown in figures 6 and 7 and as has been mentioned the fusion of the fibrin elements occurs very readily and various stages in the process can be obtained culminating in the formation of a dense and apparently homogeneous mass of fibrin from the loose fibrin net.

OBSERVATIONS ON THE PREPARED MATERIAL OF THE PLASMA CLOT

In preparing the material for histological study, the clot was preserved in Zenker, sectioned at 10 microns and then stained with Mallory's connective tissue stain modified by Mall.[13] From the study of the prepared material of the plasma clot it is clear that the structure of these permanent preparations corresponds exactly to what has been observed and noted above in the study of the fresh plasma clot with the dark field.

The structure of a permanent preparation of a typical undisturbed clot is shown in figure 9. In this preparation we have a "crystalline

[11] Howell: The clotting of blood as seen with the ultramicroscope. This Journal, 1914, xxxv, 144.

[12] Howell: Structure of the fibrin-gel and theories of gel-formation. This Journal, 1916, xl, 536.

[13] Mall: On the development of the connective tissues from the connective-tissues syncytium. Amer. Journ. Anat., 1901–02, i, 338.

gel" and the fine filamentous fibrin elements of the clot can be noted. These filaments are intermeshed and cohere with each other where they are in contact to form a fine irregular net. Scattered through the interstices of the net and also lying on the fibrin elements, in some instances, are the minute round particles which form during the process of clotting and which at that time exhibit a vigorous Brownian

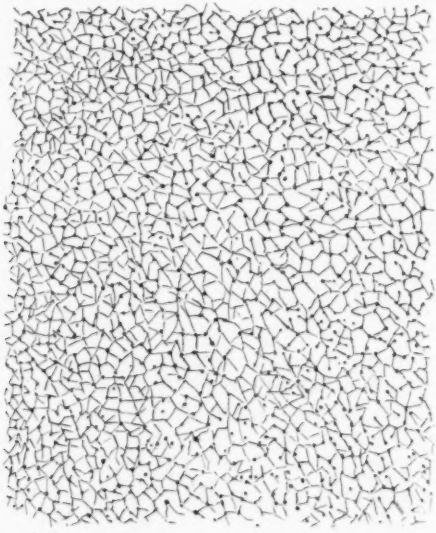

Fig. 9. Permanent preparation of plasma clot of the frog. × 1005. (Objective; Zeiss apochromatic immersion 2 mm. comp. ocular 8.) Material preserved in Zenker and stained with Mallory's connective tissue stain. The figure shows the structure of a typical fibrin net in a plasma clot. Distinct fibrin elements consisting of rods and filaments are present. Also numerous small round particles are to be seen resting on the rods and in the interstices of the net. (Cf. figure 3 which shows the same type of structure in a fresh clot.)

movement. A comparison of this figure with figure 3 shows clearly that the structure, as found in the fresh and preserved plasma clot material, corresponds very closely.

It has been noted in the previous section that when tension is applied to a fibrin clot, a transformation occurs and fibers are formed which ramify through the clot following, in general, the lines of tension. A study of the permanent preparations made from preserved clots

which have been subjected to mechanical factors of various kinds also shows this transformation in the structure very clearly. In such cases the typical fibrin net of the clot, as shown in figures 3 and 9, is largely lost and a new fibrous tissue results in which wavy bundles of fibers have formed. This transformation of the typical clot can be brought about in various ways, some of which have been previously noted in the study of the fresh clots. One of the most satisfactory methods for bringing about this transformation of the plasma clot for permanent preserved preparations is by suspending it from a silk thread for a few hours. By revolving a thread, which is held in contact with a piece of fresh clot, some of the fibrin elements will become entangled and as a result the clot will become firmly attached to the thread. The ends of the thread can then be fastened to supports inside a moist chamber and the clot left suspended and subject to a constant tension as a result of the gravity pull. This constant tension to which the suspended clot is subjected can be augmented by the use of fine lead shavings. It was found that if the lower surface of a suspended clot came in contact with the lead shavings, they would adhere to the clot by surface tension. Such a clot would thus have several grams weight added to it and the tension from gravity would be correspondingly greater. After the clot had been suspended in the moist chamber for the determined time, the lead chips were removed and the clot preserved in Zenker as usual for the permanent preparations. This method of exerting a continuous and uniform tension on a clot was found to be very satisfactory and was made use of in a number of experiments, some of which are noted below.

In figure 10 a portion of a plasma clot preparation is shown, at a magnification of 525 diameters, which was suspended with lead chips attached as noted above, for three hours. The resulting transformation of portions of the clot from that found in a typical fibrin net has been due to the tension exerted by the gravity pull. A study of this preparation shows that the transformation has resulted in the formation of bundles of wavy fibers. These are shown in the lower left hand corner of the figure. From these fibrous bundles, the individual fibrils composing them can be traced to the regions of the preparation which are untransformed and retain the typical fibrin net structure of a plasma clot. Various gradations of structure can be found ranging from a typical fibrin net to a fibrous tissue. It is clear that the fibers which have formed, as shown in the figure, are the result of a fusion and consolidation of the fibrin elements and, in general they run parallel to the line of tension exerted by the gravity pull.

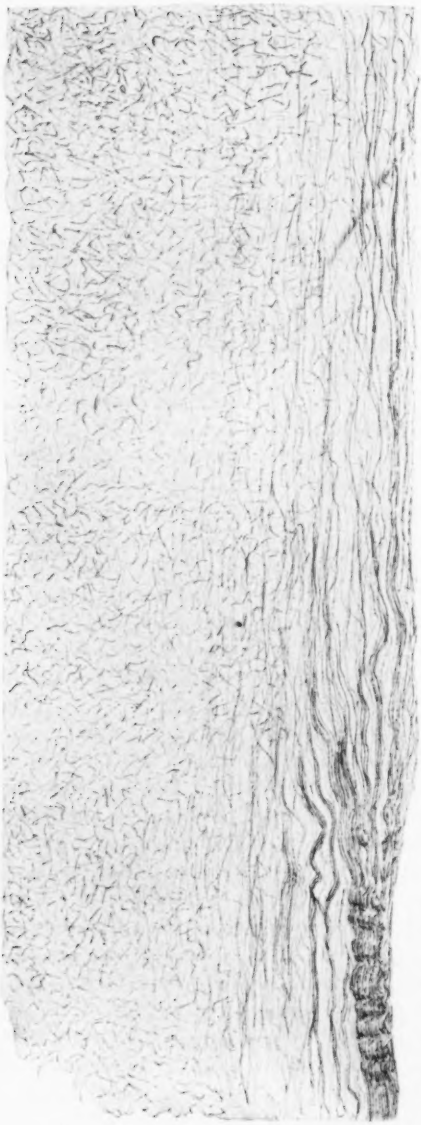

Fig. 10. As in figure 9. × 525. (Comp. oc. 4.) This figure shows a partial transformation of a portion of a plasma clot into the fibrous tissue. This transformation is due to a fusion and consolidation of the fibrin elements as a result of tension. The clot was suspended for four hours in a moist chamber subject to a gravity pull. The transformation is most complete in the lower left hand corner. In the upper portion of the figure the clot is normal in structure and distinct unfused fibrin elements are to be seen. (Cf. figure 7 which shows a partial transformation of a fresh clot.)

It is possible to carry the transformation of the plasma clot to a much more complete development and under suitable conditions of tension to get a fibrous tissue as shown in figure 11. This figure is from a portion of a clot preparation which was suspended from a silk thread in the same manner as the preparation shown in figure 10, but in this case the tension exerted by the gravity pull was allowed to act

Fig. 11. As in figure 9. × 525. (Comp. oc. 4.) This preparation shows an almost complete transformation of the plasma clot into the new fibrous tissue. In this preparation the clot was suspended for 18 hours in a moist chamber and as a result of the tension the fibrin elements have become fused and consolidated to form heavy fibrous bands. The transformation is most nearly complete in the lower portion of the figure.

for a much longer time (18 hours). Under these conditions it is possible to transform almost completely the plasma clot into a fibrous tissue having very heavy fibrous bundles in some of which, where the tension has been the greatest, there has been a fusion of the fibrin elements so that the individual fibrillae composing them can no longer be seen. This figure is also of great interest because it shows various

gradations in the transformation ranging from a typical fibrin net to a fibrous tissue with the heavy bands of fibers. In such a preparation it appears that the interior regions of the clot are subjected to a lesser degree of tension during the suspension than are the outer regions. As a result we find that the regions in which the most complete transformation takes place are toward the periphery of the suspended clot. Such is the case in the present preparation. Below, the edge of the figure marks the edge of the suspended clot. It will be noted that it is in this region that the most complete formation of the new fibrous tissue has occurred and heavy fibrous bundles have formed in which, in a number of cases, such a complete fusion of the fibrin elements of the clot has taken place that it is not possible to distinguish fully the individual fibrillae which form them. As we go toward the top of the figure, into the interior of the preparation, the fibrous bundles become less heavy, the fusion having been less complete, and it is possible to note the fine wavy fibrillae of which the fibrous bundles are formed. Near the top of the figure a region is present in which the fibrin net retains more or less of its typical, normal structure and distinct unfused fibrin elements are to be seen. It is clear from this preparation that, by the suspension of a fibrin clot, gradations in structure can be obtained ranging from a typical fibrin net to a heavy fibrous tissue and that all of these various stages have arisen directly by a more or less complete fusion of the elements of the fibrin net as the result of a mechanical factor.

From the various experiments that have been given and the study of both fresh and prepared material, it appears evident that a normal frog plasma clot is in a condition from which it can be readily changed or transformed into a new fibrous tissue by the action of a mechanical factor such as tension or pressure. The degree of the transformation apparently depends, first, upon the condition of the plasma. The experiments show that plasma of different animals varies a great deal. In some cases the clot will form very quickly and will be quite firm. Again it will form very slowly and will be much more fluid. These differences are due, in all probability, to the amount of fibrin formed in the plasma. Contact with living, injured tissues causes the firmest clots to form from the plasma. In the second place, the degree to which the transformation of the clot takes place is dependent upon the strength of the mechanical factor employed and the length of time that it was permitted to act. By varying these conditions it is possible to control the degree of fusion and therefore, the type of fibrous tissue which will be formed from a clot.

COMPARISON OF THE NEW FIBROUS TISSUE WITH CONNECTIVE TISSUE OF THE TADPOLE

The study of the clots in both the fresh and preserved material has given the complete history of the new fibrous tissue and shown conclusively that it results from a direct transformation of the fibrin elements of the clot. In conclusion, it may be well to make a comparison of the morphology of the new fibrous tissue and the normal young connective tissue of tadpoles, as shown by a histological study of the prepared material. Considerable work along this line has already been done by the author and mention of the results obtained was made in the two previous papers. These results show, in brief, that the new fibrous tissue, formed in both living cultures of frog tissues and in experimental wounds made in the skin of frogs, is apparently identical in its morphological structure with various types of normal connective tissues and scar tissues. It was also mentioned that the new fibrous tissue in its staining reactions and in digestion tests with pancreatin gave the same results as young connective tissue from tadpoles.

The same statements would apply to the present study of the fibrous tissue formed by direct transformation of the plasma clots as the result of a mechanical factor. The close resemblance in structure between the new fibrous tissue and normal connective tissue from the tadpole can be clearly shown by photomicrographs of the two tissues. In figure 12 is shown a photomicrograph, at a magnification of 940 diameters of the new fibrous tissue formed from a plasma clot. This is a portion of the same preparation which is shown in figure 11. Wavy bundles of fibers which are made up of long wavy fibrillae are to be noted. These have formed by a fusion of the fibrin elements as before described. Areas of the clot which are more or less undifferentiated and retain more or less of the typical fibrin net structure are also to be seen in the figure.

In figure 13 is shown a photomicrograph of a preparation of normal, young connective tissue taken from a tadpole. This figure is also at a magnification of 940 diameters and the material was prepared in the same manner as figure 12. Here again the fibrous bundles composed of long, wavy fibrillae are to be seen. Areas of the preparation are also to be noted in which a more or less undifferentiated condition of the ground substance persists. These areas also correspond closely in structure to the undifferentiated areas of the fibrin clot as shown in

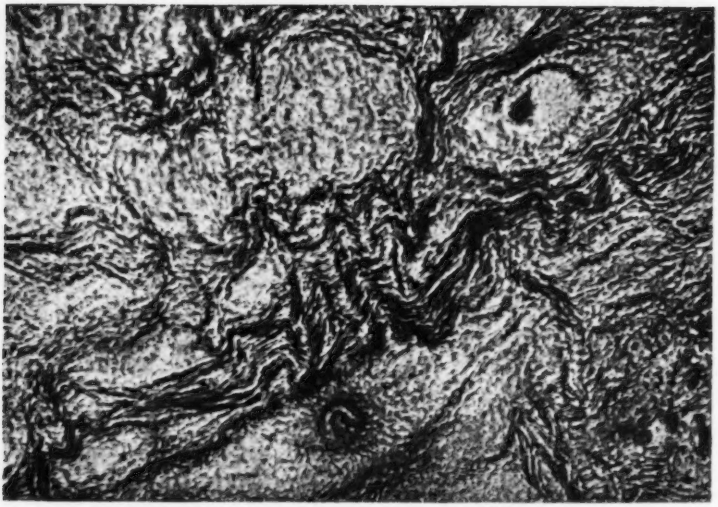

Fig. 12. As in figure 9. × 940. This photomicrograph shows a portion of a transformed plasma clot at a higher magnification than the drawing shown in figure 11. It should be compared with figure 13 to note the similarity in the histological structure between the fibrous tissue formed from a plasma clot and regular connective tissue from a tadpole.

Fig. 13. Permanent preparation of connective tissue from near the spinal cord of a tadpole. × 940. This photomicrograph is for comparison with figure 12. Both were preserved in Zenker and stained with Mallory connective tissue stain.

figure 12. Very few cells of any description are present in the preparation of connective tissue and a careful study of it and other similar preparations taken from various regions of tadpoles in different stages of development would appear to give very good evidence that the fibrillae of such connective tissues may have arisen by a transformation of a structureless ground substance in the same way as the fibrillae are formed in the plasma clots by a fusion of the fibrin elements.

It is sufficient for the present to emphasize the similarity[14] in histological structure between the fibrous tissue in a transformed plasma clot and normal connective tissue taken from various regions of the tadpole. The question of the genesis of the connective tissues in the frog will, it is expected, be fully considered in a later paper.

In brief, then, the study of the prepared material of the transformed plasma clots gives results which are in perfect agreement with the observations made on the fresh material with a dark field and as a result of all the experiments it is conclusively shown that a fibrin net of a normal clot of frog plasma can, by the action of various mechanical factors and the resulting fusion and consolidation of the separate

[14] R. A. Lambert, in the Proc. of the Soc. for Exper. Biol. and Med., 1916, xiv, 5, states that he has observed the formation of "coarse fibrils sometimes wavy in character . . . . in clotted fowl, human and rabbit plasma, as well as in frog plasma studied by Baitsell". Also that "The formation of these coarse fibrils is evidently the result of the contraction of the clot with the fusion of many of the delicate fibrin threads." . . . . "In human pathological material one sees a similar formation of coarse fibrils wherever fibrin in any quantity is deposited as for example, in fibrinous pleurisy, peritonitis, thrombi, pneumonic exudate, etc." . . . . "In their physical character the newly formed fibrils resemble collagen fibrils . . . . " However, Lambert feels that the resemblance is a superficial one and that it is not of importance in a differential study.

In my work thus far, I have not been very much concerned as to whether this new fibrous tissue retains its fibrin character, i.e., as to whether its chemical composition remains unchanged, although so radically changed in structure, as to the fact that a fibrin clot is capable of being transformed into a fibrous tissue in which one can observe, both in the fresh and prepared material, wavy bundles of fibers such as are regarded as characteristic of connective tissues. This new tissue will stain blue with Mallory's connective tissue stain, either regular or modified by Mall, the same as does regular connective tissue. It appears that very little is known at present concerning the chemistry of embryonic connective tissues and it would seem that further knowledge along these lines is essential before questions regarding the chemical transformation of the new fibrous tissue and the relation it bears both to fibrin and to collagenous tissue can be fully settled.

fibrin elements, be transformed into a fibrous tissue which in its morphological structure, to say the least, is apparently identical with normal connective tissue of the frog.

DISCUSSION

We have as a result of the present experiments the formation of a fibrous tissue from a plasma clot in direct response to the influence of mechanical factors of various kinds. The experiments have eliminated all possibility of the new fibrous tissue being formed by cellular action. The structure of the new fibrous tissue as shown by a histological study of the prepared material closely resembles that of regular connective tissue.

The fact that the new fibrous tissue is similarly formed in wounds,[15] i.e., by a direct transformation of the fibrin clot, where it functions as a regular connective tissue for a time at least, gives clear evidence that the transformation of the plasma clot is not an artifact, which occurs merely in tissue cultures and in clots which have been subjected to various external influences, but it is a reaction which evidently plays an important part in wound healing in the frog. We have no conclusive evidence at present that the transformation of the plasma clot into the new fibrous tissue is more than a radical morphological change. It is difficult to see how a chemical transformation of the protein fibrin net into a collagenous connective tissue could be brought about and consequently there is the possibility that the new fibrous tissue serves only temporarily as a connective tissue and is later replaced by a permanent tissue formed by a cellular action of some sort. On a priori grounds, however, there would appear to be no good reason for supposing that a tissue such as the new fibrous tissue which is typical in its structure and which functions normally in wounds would ever be replaced by another tissue.

Since then, in the fibrin net of the blood and lymph clots of the frog, we have a material capable of being formed into a fibrous tissue which in its morphological structure is apparently identical with regular connective tissue and which, at least temporarily, functions as such in wounds, it would appear reasonable to expect that a reaction of this kind plays an important part in the ontogeny of the individual in the formation of the various connective and supporting structures. The well-known fact that they, in general, are laid down in exact

[15] Baitsell: Op. cit., 1916.

correspondence to the definite stresses of the organism leads to the conclusion that in their formation some arrangement must have been present which would respond to the various mechanical factors introduced during development, such as has been shown by these experiments to be the case with the plasma clot. The generally accepted view of the intracellular origin of the connective tissues does not give any adequate explanation of this fact.

SUMMARY

The results of the present experiments give the complete history of the new fibrous tissue which, as shown by the author in two previous papers, forms in living cultures of adult frog tissues and in experimental wounds made in the skin of frogs by a direct transformation of the plasma clot due to a fusion and consolidation of the fibrin elements.

The observations made with a microscope equipped with a condenser for dark field illumination, on the fresh plasma of the frog while it clotted, show that normally the fibrin is deposited in the form of definite rods or filaments which intermingle and cohere to form a network, ("crystalline gel"). In old plasma a clot forms but the fibrin elements which are deposited during the process are too small to be seen as distinct units ("structureless gel").

The clot when formed can be acted upon by various mechanical methods which will produce tension or pressure and as a result it will lose its normal structure and become directly transformed into the new fibrous tissue composed of long wavy fibrillae which unite to form heavy fibrous bundles. The extent of the transformation can be controlled by the strength of the mechanical factor employed and the time it is permitted to act. The fibrous tissue which is thus formed is apparently identical in its structure with various types of regular connective tissues.

It is suggested that since blood and lymph clots of the frog have, in the elements of the fibrin net, a material which is capable of being transformed into a fibrous tissue in response to various mechanical factors, a reaction of this kind may play an important part in the formation of the connective and supporting tissues in the development of the individual.

THE VOLUME OF THE BLOOD DURING HIBERNATION AND OTHER PERIODS OF THE YEAR IN THE WOODCHUCK (MARMOTA MONAX)

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INTRODUCTION

In a previous paper (1) by one of us certain changes in the number of blood corpuscles, the amount of hemoglobin and the specific gravity of the blood during hibernation and other stages in the annual cycle in the woodchuck were reported. Such data while instructive are not of full value without a knowledge of the changes in the quantity of blood.

During the last sixty years the blood volume of man and a number of lower animals has been the subject of much study by a variety of methods, but apparently no data are on record regarding the volume of blood during hibernation.

Dubois (2) bled an active marmot through the cut carotid artery till the blood stopped of its own accord, and got 43.26 grams per kilogram of body weight. Similar treatment of a hibernating animal with a rectal temperature of 8°C. yielded only 31.30 grams of blood per kilogram. This animal did not die, however, and the next day, at which time it had a rectal temperature of 35.4°C., another 28.75 grams per kilogram of body weight was obtained by hemorrhage. But this gives no valid idea in regard to the relative amount of blood in the two animals because of the long interval intervening between the bleedings in the case of the hibernating animal and the probable influx of lymph in the meanwhile, and because there was apparently no effort made to estimate the amount of blood remaining in the animals. The present investigation was therefore carried out in the hope of getting some idea of the blood volume during the various seasonal conditions exhibited by an animal subject to a long hibernating period.

METHOD

The woodchucks used in this study were captured in the vicinity of Ithaca, New York, and kept in the artificial burrows described elsewhere (3) or under similar conditions. During the summer the animals were kept until they had become sufficiently accustomed to their surroundings to eat and drink freely. In the autumn, before they naturally retire, a larger number, to be sacrificed during hibernation, was obtained and fed till late in November or early in December, after which no more food or water was available. Some were kept till after hibernation. One of these (no. 60-SIII) was given food and water from April 5, 1916, until April 24, 1916, when it was sacrificed. The other animals (the last five) which were investigated in the spring were caught near Ithaca and fed a few days before being operated upon. Animal no. 50-SIII was found during the hibernating period under a pile of lumber near by and was kept in the house over night, during which time it awakened and became active. In this condition it was brought to the laboratory and on the following day used in this experiment.

The technique employed was essentially that used by Dreyer and Ray (4) which is undoubtedly the most accurate method so far devised.

The animals were rapidly anesthetized with ether or chloroform or both. In the case of dormant animals it was found best to start out with chloroform since its more powerful action made it possible to induce anesthesia without waking the animal up or producing noticeable excitement. In a few cases excitement of the dormant animals was prevented by giving chloretone per rectum one hour preceding the operation. On account of the possible effect which the greater activity attending excitement might have on the blood volume, these precautions seem necessary. After the urine was expelled by pressure, the body weight was recorded. A cannula was next inserted in the right jugular vein through which oxygenated Locke's fluid, warmed or cooled to the body temperature of the animal, could be perfused by gravity. By working with blunt instruments through the same median incision, the left carotid artery was isolated and into it inserted a paraffined cannula connected with a short rubber tube. About ten drops of blood were allowed to escape into distilled water in order to wash out the cannula and rubber tube. A little over 1 cc. of blood was then collected in a small paraffined vessel and from this exactly 1 cc. was taken by means of an accurately calibrated pipette. This

1 cc. was expelled into distilled water, made up to 100 cc. and constituted the standard against which the amount of hemoglobin in all the rest of the blood was later calibrated. Then nearly 6 cc. of blood was collected in a specific gravity bottle and weighed, after which this blood was returned to the distilled water in which the rest of the blood was being received. Finally oxygenated Locke's solution was allowed to enter the jugular at the same rate as the blood was allowed to escape through the carotid so as to maintain about the same amount of liquid circulating in the vascular system. Respiration usually continued till the perfusion liquid escaping from the animal was nearly colorless. The heart beat continued for some time after respiratory movements ceased. When the heart failed, the abdomen was opened along the median line and the contents of the stomach and intestine carefully squeezed out through openings made here and there along the gut. These openings were then ligated again so that any bile which might be poured into the intestine and the slight amount of feces which discolored the cœcum, especially, would not get into the blood washings. A large cannula was then inserted into the base of the aorta through an incision in the chest wall near the median line and through the left ventricle. Normal salt solution was then forced into the arteries and allowed to escape from the right atrium. In this way the slightly colored perfusion liquid in the vascular system was washed out. This flushing was continued for some time after the liquid came out perfectly clear. Since this washing out of the vessels sometimes caused liquid to accumulate in the stomach and intestine, the gastric and intestinal contents were first removed in order to get the proper weight of such contents. Finally a thorough examination was made to make sure that no blood was left on account of some unforeseen defect in the technique, such as embolism. From a recent paper by Rous and Robertson (5) it appears that after direct perfusion, the spleen and bone marrow still contain blood, although in all other organs the blood comes away completely. The amount of blood left in the spleen and bone narrow must be so small as to be negligible.

Nearly all the blood was collected in a sufficiently large quantity of water to cause immediate laking. The small amount of blood washed out with the salt solution was laked with saponin and added to the main mass of blood. The total volume of diluted blood (10 to 20 liters) was measured by means of narrow necked volumetric flasks of one to several liters capacity. All the washings were thoroughly mixed in a large glass vessel. A sample was then saturated with CO

and filtered, and by means of a colorimeter of the Duboscq type compared with the standard solution containing blood diluted one hundred times similarly treated with CO and filtered.

The total volume of blood was calculated and then multiplied by the specific gravity to obtain its total weight.

RESULTS

Table 1 gives the results obtained together with related data.

The data show that the weight of the blood in forty-four woodchucks (seventeen males and twenty-seven females), weighing from 813 grams to 3116 grams, varies from 8.3 per cent to 4.4 per cent of the reduced body weight. These values are very close to those given for the rabbit (6). It is seen that the percentage of blood during July is in the neighborhood of 7 per cent while in November and early December before dormancy has set in it has fallen to a minimum of about 5 per cent. This low percentage is thus reached when the animal has a maximum amount of fat stored within the body, and the decrease from the larger percentage found in July is undoubtedly due to obesity. This would indicate that adipose tissue is not nearly so vascular as the body as a whole. That an increase in obesity decreases the relative percentage of blood has been shown by several workers. Ranke (7) found in rabbits that poor animals weighing up to 1300 grams contained on an average of 5.5 per cent of blood while fat animals weighing over 1400 grams contained only 3.3 per cent. Haldane and Smith (8) by means of the CO method found that while in general the weight of the blood in man was nearly 5 per cent, that of a very fat man was only 3.35 per cent. In a more recent paper on the subject of blood volume, Keith, Rowntree and Geraghty (9) by a new method (the injection of a known quantity of vital red) show that in man the blood of the average normal individual represents 8.8 per cent of the body weight, but in obese individuals the average was found to be less than three-fourths of the normal volume. One individual, e.g., had only 51 cc. of blood per kilogram of body weight, whereas the normal volume by this method is 85 cc. per kilogram of body weight. The woodchuck becomes exceedingly fat as winter approaches and this is undoubtedly the explanation of the decreased percentage of blood.

In looking at the figures showing the percentage of blood it is necessary to consider the size of the animal. It was originally intended to use only large animals of about the same size in order to make com-

TABLE 1

DATE OF DETERMINATION	ANIMAL No.	Sex	Rectal temperature. C.	ACTUAL BLOOD VOLUME	THEORETICAL BLOOD VOLUME K = 1.7	MODIFIED THEORETICAL BLOOD VOLUME	DEVIATION OF ACTUAL FROM THEORETICAL VOLUME	REDUCED (NEt) BODY WEIGHT	WEIGHT OF BLOOD	BLOOD AS PER CENT OF BODY WEIGHT	DAYS SINCE FOOD WAS LAST AVAILABLE	REMARKS
				cc.			per cent	grams	grams			
Midsummer (July) Fed												
July 8, 1915	5-SIII	♀	36.1	187.0	176.5		+ 5.9	2,757	198.9	7.2		Active. In good condition
July 9, 1915	6-SIII	♀	38.4	165.0	162.8		+ 1.4	2,464	174.4	7.1		Active. In good condition
July 20, 1915	12-SIII	♂	36.7	178.0	173.4		+ 2.7	2,690	187.0	7.0		Active. Poorer than above two
July 29, 1915	17-SIII	♀	36.7	176.0	176.6		− 0.3	2,760	185.5	6.7		Active. In good condition
July 6, 1916	72-SIII	♂	36.0	179.5	186.2		− 3.6	2,970	190.4	6.4		Active. In good condition
July 7, 1916	74-SIII	♀	37.0	153.2	151.3		+ 1.3	2,227	162.1	7.3		Active. In good condition
July 7, 1916	75-SIII	♀	38.0	151.0	154.4		− 2.2	2,290	159.5	7.0		Active. In good condition
July 8, 1916	76-SIII	♀	37.0	162.0	173.8		− 6.8	2,700	171.4	6.3		Active. In good condition
July 8, 1916	77-SIII	♀	38.0	168.9	160.8		+ 5.0	2,422	178.8	7.4		Active. In good condition
Midsummer (August) Fed												
Aug. 26, 1915	29-SIII	♀	36.9	137.2	151.5		− 9.4	2,230	146.0	6.5		Active. Fatter than above group
Aug. 2, 1916	78-SIII	♀	37.2	135.4	169.2		−20.0	2,600	143.6	5.5		Active. Very fat
Aug. 2, 1916	79-SIII	♀	37.0	78.3	95.2		−17.8	1,170	82.0	7.0		Active. Fat. Not adult
Just before hibernation. Fed												
Nov. 6, 1915	37-SIII	♀	36.5	100.8	125.9		−19.9	1,725	106.9	6.2		Active. Fat
Nov. 20, 1915	39-SIII	♂	36.0	88.5	126.2		−28.9	1,730	93.5	5.4		Not so active. Very fat. Not adult
Nov. 23, 1915	40-SIII	♀	37.0	82.7	120.4		−31.3	1,622	87.4	5.4		Not so active. Very fat
Dec. 1, 1916	1-SIV	♂	35.0	90.0	146.9		−38.7	2,138	94.7	4.4		Not so active. Very fat
Dec. 2, 1916	2-SIV	♀	36.0	133.7	170.1		−21.4	2,620	142.0	5.4		Not so active. Very fat
Dec. 2, 1916	3-SIV	♂	36.0	131.9	173.4		−23.9	2,690	139.1	5.2		Not so active. Very fat
Dec. 3, 1916	4-SIV	♀	36.0	76.2	122.5		−37.8	1,660	79.9	4.8		Not so active. Very fat
Dec. 3, 1916	5-SIV	♂	35.0	141.2	192.7		−26.7	3,116	149.0	4.8		Fattest of entire lot.

During hibernation. No food or water available

Dec. 18, 1915	42-SIII	♀	9.0	108.2	145.1	141.7	−23.6	2,100	114.2	5.4	25	Dormant. Much fat present
Dec. 21, 1915	43-SIII	♂	19.0	131.4	165.6	161.5	−18.6	2,525	140.0	5.5	28	Sluggish. Much fat present
Feb. 7, 1916	48-SIII	♂	6.0	61.5	73.3	67.9	− 9.5	813	65.3	8.0	76	Dormant. Very poor
Feb. 8, 1916	49-SIII	♂	23.0	98.5	120.1	110.8	−11.1	1,615	104.4	6.5	77	Sluggish. Much fat present
Feb. 25, 1916	50-SIII	♀	35.0	147.4	184.6	167.9	−12.2	2,936	157.2	5.4	90(?)	Awake 1 day. Exceptionally fat
Feb. 26, 1916	51-SIII	♀	7.0	87.4	121.1	109.0	−19.8	1,635	92.5	5.7	95	Dormant. Some fat left
Mar. 3, 1917	6-SIV	♀	15.0	81.6	95.0	86.5	− 5.7	1,167	86.5	7.4	90	Dormant. Some fat left
Mar. 4, 1917	7-SIV	♂	16.0	72.2	87.8	79.6	− 9.3	1,045	76.4	7.3	91	Dormant. Some fat left
Mar. 17, 1917	8-SIV	♂	14.0	103.9	129.8	114.8	− 9.5	1,800	110.1	6.1	104	Dormant. Some fat left
Mar. 18, 1917	9-SIV	♂	12.0	97.9	124.3	111.4	−12.1	1,695	104.0	6.1	105	Dormant. Very little fat left

After hibernation and before being fed

Apr. 1, 1916	55-SIII	♂	24.0	78.1	105.8	91.4	−14.6	1,355	83.0	6.1	128	Waking up. Some fat left
Apr. 3, 1916	56-SIII	♂	34.0	161.0	150.5	129.3	+24.5	2,210	170.9	7.7	130	Awake several weeks. Very poor
Apr. 14, 1917	10-SIV	♂	34.0	98.0	105.0	89.5	+ 9.5	1,340	104.2	7.8	132	Awake 4 days. Poor
Apr. 14, 1917	11-SIV	♀	34.0	94.8	110.3	94.2	+ 0.6	1,435	100.7	7.0	132	Awake several weeks. Some fat
Apr. 15, 1917	12-SIV	♀	34.0	64.3	75.8	64.8	− 0.8	852	68.4	8.0	133	Awake weeks. Poor. Not adult
Apr. 15, 1917	13-SIV	♀	36.0	79.8	105.5	90.1	−11.4	1,350	84.8	6.3	133	Awake weeks. Much fat left
Apr. 16, 1917	14-SIV	♀	37.0	82.2	95.8	81.9	+ 0.4	1,180	87.3	7.4	134	Awake several weeks. Very poor

After hibernation and after feeding three to six weeks

Apr. 22, 1916	59-SIII	♂	36.0	110.2	128.5	119.8	− 8.0	1,775	115.8	6.5		Active. Very little fat present
Apr. 24, 1916	60-SIII	♀	37.5	76.9	95.5	89.9	−14.5	1,175	80.7	6.9		Active. More fat than in above
Apr. 28, 1916	61-SIII	♀	35.0	91.3	114.5	108.2	−15.6	1,511	96.9	6.4	2(?)	Active. Little fat. Post partum
May 7, 1916	64-SIII	♀	37.0	135.0	124.3	119.3	+13.2	1,695	141.5	8.3		Active. Very poor. Post partum
May 10, 1916	66-SIII	♂	37.0	165.3	160.7	155.0	+ 6.6	2,420	174.7	7.2		Active. Very poor. No fat
May 12, 1916	67-SIII	♀	38.0	113.2	124.1	120.1	− 5.7	1,690	118.8	7.0		Active. In good condition
May 13, 1916	68-SIII	♀	38.5	113.6	130.3	126.3	−10.0	1,810	119.2	6.6		Active. In good condition

parisons simpler, but it was found necessary to depart from this rule on account of the scarcity of available animals. It is evident from the analysis of the literature by Dreyer and coworkers (6) that smaller animals have relatively a greater blood volume than do larger ones even though the animals are in the same nutritional condition. In eight dogs involved in experiments reported more recently by Scott (10), there is only one exception to this rule. While in most cases where woodchucks have been sacrificed at about the same period of the year this rule holds, there appear to be several exceptions. Some of these are undoubtedly due to variations in nutritional condition so that body weight is not a true criterion of the size of the animal. If data were available body length, on account of being less susceptible to variations (11), would undoubtedly be a much better criterion of size. As a rule, however, woodchucks of the same weight and killed about the same time usually check very closely.

Dreyer and coworkers (12) have further shown that the blood volume is a function of the surface area of the animal and may be expressed by a formula based upon Meeh's (13) formula for determining the surface area of the body from its weight. The formula as finally used (14) in the case of warm blooded animals is

$$B = \frac{W^{.72}}{K}$$

where B is the blood in cubic centimeters; W, the weight of the animal in grams and K, a constant to be calculated for each species of animal by experimental determination of the blood volume and substituting this for B in the equation

$$K = \frac{W^{.72}}{B}.$$

Assuming that the Dreyer-Ray formula is also true for the woodchuck just as it is for the six species of mammals (tame rabbit, wild rabbit, hare, mouse, wild rat, guinea pig) especially investigated and including wild animals direct from their native habitat as well as domesticated ones, woodchucks of different sizes may be rendered directly comparable by calculating the theoretical blood volume by means of the above formula and comparing the resulting volume with the actual blood volume. This has been done here. The average value of K for the woodchucks killed in July was found to be 1.7. This value of K has been used throughout in all calculations of the theoretical

blood volume, thus making the July animals the standard. These particular animals were chosen as the basis of comparison for the reason that they seemed to be in good nutritional condition but not excessively fat. In the spring these animals are recovering from months of inanition and later in the summer and autumn they are storing up large quantities of fat—two rather unusual conditions. The difference between the theoretical volume and the volume found experimentally has been tabulated as per cent of the theoretical blood volume, with a plus or minus sign preceding to indicate whether the actual volume is above or below the theoretical volume. Normally, according to Dreyer and Ray (14), there may be a variation of 12 per cent from the calculated volume.

It is clearly evident from an examination of the column showing the deviation of the actual from the theoretical volume that even in August, but especially later in the year or just before hibernation, the actual blood volume is relatively far below that found in July. The decrease late in November and early in December varies from 21 per cent to nearly 39 per cent. Three of the seven included in this period had a volume more than 30 per cent below the theoretical volume The very fattest animal (5-SIV) did not, however, have the lowest relative volume.

With the progress of hibernation, during which time the animal is in a condition of complete inanition, the percentage of blood increases so that after about three months of more or less dormancy, the deviation of the actual from the theoretical volume is within normal limits though on the negative side, i.e., the volume is slightly less than the formula calls for. During this period it is especially necessary to take into consideration the size of the animal, for while the percentage of blood is over 7 per cent in two cases and in one case over 8 per cent, these three animals are unusually small and reference to the column showing the deviation of the actual from the theoretical blood volume indicates that the blood volume is slightly subnormal.

In making the calculations for animals sacrificed durng the hibernating period and those that have awakened from winter sleep but have not had access to food and in fact for all animals that are under weight, it seemed desirable to make a correction in the theoretical blood volume as calculated from the weight of the animal when sacrificed because the formula calls for a higher percentage of blood in lighter animals than in heavier ones. At the close of hibernation the animals have lost about one-third of their weight and hence the formula applied to these

emaciated animals calls for a percentage of blood equal to that of an animal only two-thirds as large. Take, for example, the last animal (9-SIV) in the hibernating group which weighed only 1695 grams (net) when killed. This animal before hibernation weighed in the neighborhood of 2500 or 2600 grams. In other words, it is an animal of about the same size as 2-SIV (one of the group investigated just before hibernation). The column of theoretical blood volumes shows that the formula in this latter case (2-SIV) calls for 170.1 cc. of blood, which is 6.49 per cent of the number of grams of the body weight (2620), while in the other case (9-SIV) the theoretical blood volume is 124.3 cc., which is 7.33 per cent of the number of grams of body weight (1695). If the relative blood volume remained unchanged during winter sleep, i.e., decreased at the same rate as the body as a whole, then the volume of blood at the end of hibernation would represent 6.49 per cent (not 7.33) of the number of grams of body weight, and 6.49 per cent of 1695 is 110. The theoretical blood volume with which we should compare the actual blood volume of animal 9-SIV is thus 110 cc. and not 124.3 cc. (the figure found in the column of theoretical blood volumes), which is 13 per cent too great. In a similar manner a modified theoretical blood volume has been calculated for each of the animals considered to be under weight. The column of modified theoretical blood volumes in table 1 thus gives as a basis of comparison in the case of the above animal (9-SIV) 111.4 cc., or about what was just determined above (110 cc.). The reason why 111.4 is used and not 110 cc. is that the loss of weight was taken in this case as exactly 32.5 per cent of the body weight for reasons to be explained below.

The basis for calculating the modified theoretical blood volume found in table 1, is taken from data obtained during 1913–1914 on a series of woodchucks kept under similar conditions. These observations are compiled in table 2. While the facts are insufficient to give details as to the rate of loss at various stages of hibernation, the data do give a fair idea of the total loss for a period of one hundred and ten days. The reduced body weight before hibernation is of necessity estimated from the gross body weight by deducting 60 to 150 grams (amounts based upon numerous subsequent findings) from the gross body weight. The gastro-intestinal contents and urine are usually more or less proportional to the size of the animal. Four animals which had been without food for one hundred and ten days and which had been awake about two weeks after hibernation, show an average loss of 35.7 per cent of the initial body weight, or a daily loss of 3.25 grams per kilogram of body

weight. With this as a basis and the assumption that the loss of weight is uniform and proportional to the number of days of inanition, a sufficiently close approximation to the weight of the animals before hibernation commenced can be made for purposes of making the correction in the theoretical blood volume. Valentine (15) reported only a loss of 18 to 23 per cent during one hundred and thirty-four days of hibernation in the European marmot. He also determined that the average daily loss per kilogram of body weight was 1.9 grams (of which 0.72 gram was fat and 0.72 gram skeletal muscle) after

TABLE 2

Showing the loss of body weight during the hibernating period

ANIMAL (SERIES 1)		BODY WEIGHT BEFORE HIBERNATION		LATER OBSERVATIONS			LOSS IN BODY WEIGHT			DAYS OF INANITION
No.	Sex	Gross	Reduced or net (estimated)	Reduced body weight	Date of observation, 1914	Rectal temperature C.	Grams	Per cent	Average daily loss per day per kilogram of body weight	(Feeding stopped December 8, '13)
		grams	*grams*	*grams*					*grams*	
5	♂	2,660	2,550	2,225	Feb. 3	8	325	12.78	2.24	57
6	♀	1,820	1,735	1,335	Feb. 16	19	400	23.05	3.29	70
7	♀	3,270	3,120	2,470	Feb. 28	12	650	20.83	2.54	82
8	♂	1,300	1,250	838	Mar. 7	9	412	32.96	3.70	89
9	♀	1,825	1,740	1,385	Mar. 14	11	355	20.40	2.13	96
10	♀	1,700	1,620	1,135	Mar. 16	32	485	29.09	2.97	98
11	♀	1,920	1,830	1,335	Mar. 18	36	495	27.05	2.71	100
12	♂	2,275	2,175	1,630	Mar. 21	32	545	25.06	2.43	103
13	♂	3,550	3,400	2,175	Mar. 25	34	1225	36.03	3.37	107
14	♂	2,650	2,540	1,730	Mar. 28	35	810	31.89	2.90	110
15	♂	2,520	2,410	1,580	Mar. 28	35	830	34.44	3.13	110
16	♀	1,450	1,390	788	Mar. 28	35	602	43.31	3.94	110
17	♀	2,250	2,150	1,335	Mar. 28	37	815	33.26	3.02	110

forty-four days and 2.18 gram (of which nearly 1 gram was fat and 0.49 gram skeletal muscle) after one hundred and sixty-three days of hibernation. From table 2 it will be seen that the average daily loss per kilogram of body weight in the woodchucks from which data is available varies from 2.13 grams to 3.94 grams. This greater daily loss in weight is undoubtedly due to a less prolonged dormancy in these animals than in the marmot observed by Valentin. Woodchucks hibernating in captivity under conditions almost identical with the natural burrow (3), have repeatedly been found awake and fairly

active for weeks during midwinter. It is also well known that in their natural habitat they are often seen awake and running over the snow-covered ground in February. They apparently once more retire to again wake up about the middle of March.

Three of the animals which had thus been emaciated by one hundred and ten days of hibernation and inanition, were fed with the result that about 11 per cent of the original weight (weight before hibernation) or about 18 per cent of the minimum weight (weight just before refeeding commenced) was regained in three weeks, when the last one of the three was sacrificed. These observations served as a basis in modifying the theoretical blood volume of the last group of woodchucks, the assumption being that they too had lost at least one-third of their weight and at the end of three weeks had regained 11 per cent of it, leaving them after three weeks of feeding about 22 per cent underweight and at the end of six weeks of feeding 11 per cent underweight.

In short, the theoretical blood volume was corrected by substituting in the formula the approximate weight of the animal before hibernation commenced, and determining what per cent of this number of grams the resulting number of cubic centimeters of blood represent and then taking this per cent of the number representing the grams of actual weight of the animal when sacrificed as the modified theoretical blood volume in cubic centimeters. In the last three groups of animals this modified theoretical blood volume was used in calculating the deviation of the actual from the theoretical volume.

Returning to table 1, it will be noted that after hibernation and before feeding commences the blood volume was in four cases out of seven back to the July proportions. One animal taken while just waking up (55-SIII) had distinctly a subnormal blood volume, as had also one other animal, while a very large animal (56-SIII) had a volume which is 24.5 per cent above. These animals are generally more emaciated than those sacrificed at earlier dates. Nearly all woodchucks that have come under our observation towards the close of hibernation or just as they are waking up, have had some fat left. One animal (13-SIV) even after being awake nearly a month still had a large amount of fat reserve, although no food or water had been available for one hundred and thirty-three days, except the excelsior in which they were kept. In the gastro-intestinal contents of this and several other animals of this group there was evidence of a small amount of excelsior having been ingested. The amount of nutrition derived from this source must be extremely limited. It will be noted that it is

the fattest animals of this group that have the subnormal blood volume. It would appear from the figures given by Valentin, and stated above, that ordinary adipose tissue is not utilized as rapidly in the earlier part of hibernation as in the later part while the reverse is true of the skeletal muscle. This undoubtedly accounts for the presence of considerable fat even after three or four months of inanition. It should be kept in mind that according to the figures given by Valentin (15), the liver loses more than half of its weight and in connection with the skeletal muscles (which lose about 30 per cent of their weight and supply about four times as much material during hibernation as does the liver) supply about half as much material, in absolute weight, as does adipose tissue during winter sleep. Other tissues also contribute, so that fat is by no means the only source of nutrition during this period of starvation. Although there is great individual variation, it is safe to conclude that in this group the blood volume may be back to the July proportions.

Finally with the resumption of feeding and consequent increase in body weight, amounting in all probability to at least 18 per cent (of the minimal weight) after feeding three weeks, the blood volume is somewhat low in five out of the seven cases and high in the other two. There are great individual differences and, as in the previous group, it is the more emaciated animals which show the highest relative blood volume. It is probably safe to say that the relative blood volume is slightly lower than in the preceding group. The interesting thing about these animals that have been fed is the blood picture. As reported by one of us (1), the erythrocytes are on an average fully 20 per cent fewer per unit volume of blood, and the percentage of hemoglobin and the specific gravity correspondingly lower than in the next preceding group. It was suggested there that this low cell count, etc., was probably due to the blood having taken up water faster than solids are acquired. This seems to be supported here although the relatively high blood volume anticipated in this group apparently does not in general exist. If reference be made to the paper (1) mentioned above, it will be found that the animal (61-SIII) with the highest cell count, per cent of hemoglobin and specific gravity, is the one with the lowest relative blood volume and, on the other hand, the animal (64-SIII) with the lowest count has the highest blood volume. No reliance, however, can be placed on these isolated cases especially where many disturbing factors are present, such as having given birth to young earlier in the spring, in one case (64-SIII), and the excitement

attending recent capture and being put in confinement, in the other (61-SIII).

So it appears that instead of an unusually rapid acquisition of water by the blood upon refeeding, it is a much slower increase in the red corpuscles and other solids which accounts for the low cell count and low specific gravity of the blood of this group.

It may be noted that two animals had been pregnant and given birth to young several weeks previously, as was evident from slightly congested areas in the horns of the uteri and well developed corpora lutea in the ovaries. This does not seem to have produced a constant effect on the blood volume since these animals represent the extremes of the group.

It is interesting to compare these observations with blood volume changes during inanition in non-hibernating animals. From the experiments of Valentin (16), Heidenhain (17), Panum (18), Voit (19) and London (20), it is clearly evident that in such animals as dogs and rabbits subjected to complete and exhausting starvation there is practically no change in the percentage of blood, notwithstanding the contrary views held by Bidder and Schmidt (21) and Chossat (22). The latter author maintained that the blood suffered a loss sometimes as great at 75 per cent of its original weight, thus coming next to adipose tissue in the extent of loss by inanition. There seems to have been no general acceptance of this view, however. Disregarding the fall in the percentage of blood with the storing of fat and the gradual return as the fat disappears during hibernation and immediately following and comparing the July animals (which are not excessively fat) with those sacrificed at the close of hibernation, the blood volume changes during inanition in hibernating animals are not very different from those of non-hibernating ones. Thus the blood tends to remain at a fairly constant ratio to the essential tissues though food is withheld even for long periods.

Among the extensive determinations of the blood volume by Keith, Rowntree and Geraghty (9) on human beings, appear two subjects with marked emaciation, one being the result of voluntary starvation. In both instances the blood volume was normal.

While the general blood volume is thus fully as resistant as the body as a whole to experimental inanition, the literature (23) indicates that the relation of solids to water in the blood usually changes—water being lost more rapidly, resulting in an increase in the specific gravity of the blood and in a higher percentage of solids. As already reported

(1) such an increase in the specific gravity of the blood during hibernation of the woodchuck does not seem to obtain unless the animal becomes more or less active, although a marked increase in the percentage of solids and in the specific gravity has been found to occur in the European marmot during winter sleep (Aeby, Dubois, Polimanti). The average specific gravity of the blood of the dormant animals (all animals sacrificed during the hibernating period with the exception of the two semi-dormant ones and the one that was fully awake) used in this study, is 1.0593, which agrees as closely as can be expected with the figure (1.0595) already reported and referred to above.

The high specific gravity of the animals which have awakened from hibernation but have not had access to food as noted in the previous report, is also fully supported here by five additional animals with an average specific gravity of 1.0625. This high specific gravity is evidently not due to the operative procedure since Mann (24) has demonstrated that light etherization does not change the specific gravity of the blood, number of erythrocytes and per cent of hemoglobin, and that an increase in the specific gravity takes place only when the anesthesia is pushed till asphyxia becomes a factor. All blood for analysis was taken usually within twenty minutes after anesthesia set in. These active animals, which have had no food or water in some cases for over four months, are virtually in the same condition as non-hibernating animals subjected to severe inanition and hence the similarity in the effect on the blood.

In the woodchuck there appears to be practically no sex difference in the blood volume as is evident from table 3. This table is compiled by picking out all the possible pairs of animals of opposite sex in which the members of each pair are approximately of the same weight and which were sacrificed during the same or corresponding season of the year and hence presumedly in about the same nutritional condition. It was possible to pick out ten pairs that satisfied these requirements. The average body weight of the males is slightly greater than that of the females with the same average percentage of blood. Since the males are slightly larger, the percentage of blood would be slightly smaller if males and females had exactly the same relative amount of blood because of the fact that larger animals have a smaller percentage of blood. The difference, however, is so small as to be insignificant since a single additional pair might easily reverse the proportion.

Dreyer, Ray and Walker (6) report that in the rabbit males have about 3 per cent more blood than females.

TABLE 3

Showing the relative blood volume in males and females

FEMALES				MALES			
No.	Date	Reduced body weight	Per cent of blood	No.	Date	Reduced body weight	Per cent of blood
		grams				*grams*	
17-SIII	July 29, 1915	2,760	6.72	12-SIII	July 20, 1915	2,690	6.98
76-SIII	July 8, 1916	2,700	6.35	72-SIII	July 6, 1916	2,970	6.41
37-SIII	Nov. 6, 1915	1,725	6.19	38-SIII	Nov. 20, 1915	1,730	5.41
2-SIV	Dec. 2, 1916	2,620	5.42	3-SIV	Dec. 2, 1916	2,690	5.17
42-SIII	Dec. 18, 1915	2,100	5.44	43-SIII	Dec. 21, 1915	2,525	5.49
51-SIII	Feb. 26, 1916	1,635	5.65	49-SIII	Feb. 8, 1916	1,615	6.47
6-SIV	Mar. 3, 1917	1,167	7.42	7-SIV	Mar. 4, 1917	1,045	7.31
11-SIV	Apr. 14, 1917	1,435	7.02	55-SIII	Apr. 1, 1916	1,355	6.13
13-SIV	Apr. 15, 1917	1,350	6.28	10-SIV	Apr. 14, 1917	1,340	7.78
67-SIII	May 12, 1916	1,690	7.03	59-SIII	Apr. 22, 1916	1,775	6.53
Average		1,918	6.35	Average		1,974	6.37

SUMMARY

1. Blood volume determinations in forty-four woodchucks (seventeen males and twenty-seven females), weighing from 813 grams to 3116 grams, showed a variation from 8.3 per cent to 4.4 per cent of the reduced body weight.

2. The percentage of blood is high in midsummer and decreases greatly (nearly 30 per cent) before dormancy sets in.

3. The percentage of blood is lowest just before hibernation begins, at a time when the animals contain a maximum amount of fat.

4. With the progress of hibernation the percentage of blood increases until toward the end of dormancy it is nearly as high as in midsummer.

5. After waking up and becoming active and before food is available, the percentage of blood is again high, though there are great individual variations. The animals are usually greatly emaciated at this time and the poorer ones have the highest percentage of blood.

6. After feeding three to six weeks the relative blood volume is probaby not quite as high as before food was available, though there is again, as in the preceding period, so much individual variation that it is somewhat hazardous to make a definite generalization. The red

corpuscle count, percentage of hemoglobin and specific gravity being much lower than at any other time, it appears that the erythrocytes and some other solids of the blood do not increase as rapidly as the blood volume during this period of restoration.

7. There is no marked sex difference in the blood volume of woodchucks.

8. Data obtained from a separate series of woodchucks which were kept in the artificial burrows used for the most part in this study, show an average daily loss in body weight of 3.25 grams per kilogram during a hibernating period of one hundred and ten days. The animals were not dormant, however, during the entire time.

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THE RELATION OF THE RATE OF THE SPONTANEOUS LIBERATION OF EPINEPHRIN TO THE RATE OF BLOOD FLOW THROUGH THE ADRENALS

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In several of our papers we have given illustrations of the fact that the rate of liberation of epinephrin (in the cat) may remain approximately constant for a wide range in the rate of blood flow through the adrenals, the concentration in the adrenal vein blood varying inversely as the flow (1). Numerous assays, incidentally accumulated, have indicated not only that this is true in one and the same animal under experimental conditions, but also that the output in different cats varies less than might have been expected (2). It has seemed worth while, however, to supplement these observations by some experiments specially directed to the question whether with such quantitative methods as have been employed an exact inverse ratio between epinephrin concentration and adrenal blood flow can be made out. It will be more instructive to reproduce a sufficient number of the tracings from a typical assay to illustrate fully the degree of sharpness which can be obtained in such observations rather than to give one or two specimens from each of the experiments. We lay stress on making multiple observations on any specimen of blood which is being assayed for epinephrin by the rabbit intestine and uterus segment method. With a single segment a very large number of observations can be carried out without loss of accuracy in the comparisons if two simple precautions be adopted: First, always comparing tracings which are not too far apart in the series; and second, repeating observations freely. It is scarcely necessary to observe that although some of the experimental conditions may be changed at will in the course of a series of observations, for example, the weight, comparison must not be made of tracings taken before with tracings taken after the change. A suitable intestine segment, far from deteriorating from long use, is often seen to improve after a time as regards constancy

of response. This does not mean that it also improves in regard to sensitiveness although segments long worked with may still be very sensitive to small concentrations of epinephrin. An exceedingly sensitive segment, however, although indispensable when the business is to detect minimal quantities of epinephrin, for example, in determining whether any liberation is taking place after section of the nerves of the adrenal (2) is not necessarily advantageous in assaying such concentrations as normally exist in blood coming from the adrenal veins. Here, constancy of response is far more important than sensitiveness. It scarcely needs to be pointed out that a segment may distinguish clearly between blood containing no epinephrin and blood containing 1: 100,000,000, and yet may not give a clear difference between two bloods containing respectively 1: 3,000,000 and 1: 3,500,000 even when the bloods are applied in all degrees of dilution. All the tracings given in figures 1 to 13, as well as others not reproduced, were obtained from one intestine segment, on which ten specimens of blood were assayed.

Experiment 1. Condensed protocol. Cat (pregnant). Weight, 3.03 kgm. Urethane, 5 grams by stomach tube. Inserted tracheal and jugular cannulae and obtained a specimen of jugular blood. Made a "short"[1] cava pocket, tying the renal, coeliac and mesenteric arteries and the abdominal aorta. Then collected the following specimens of adrenal blood.

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION	BLOOD FLOW PER MINUTE
	grams	*minutes*	*grams*
1	4.7	2	2.3
2	7.8	4	2.0
3	10.1	6	1.7
4	9.8	8	1.2
5	3.5	9	0.4

Now collected more blood from the jugular. Combined weight of the two adrenals 0.360 grams. The bloods were immediately put on ice and after two hours tested on rabbit intestine and uterus segments, an assay of the epinephrin being made on the former.

[1] Where blood is collected from the cava pocket through a cannula it is advantageous to make the pocket much shorter (by inserting the cannula higher up in the vein) than in cases where the pocket is used as a receptacle to accumulate adrenal vein blood which is then released into the circulation. In the latter case it is essential to have a capacious pocket, whereas in the former it is more important to reduce the dead space, so that overlapping of successive samples as well as the risk of clotting may be minimized.

Tracings illustrating the assay of the various adrenal blood samples on a rabbit intestine segment are reproduced in figures 1 to 7. Figure 1 shows that the fourth specimen (observation 32) has a distinctly greater effect on the segment than the second (observation 30); and that the concentration of epinephrin in the fourth specimen is somewhat greater than 1:2,700,000 (observation 36). The adrenalin (Parke, Davis and Company) was assayed by the colorimetric method of Folin, Cannon and Denis. It was shown by the uterus tests (not reproduced)

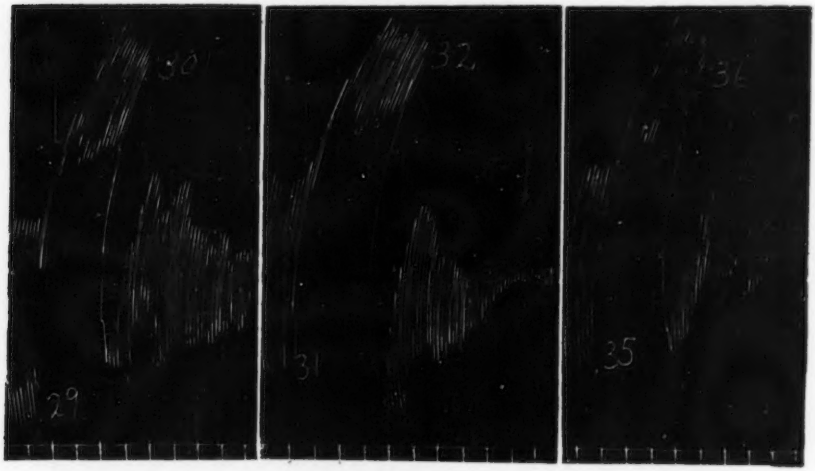

Fig. 1. At *29* and *31* Ringer's solution was replaced by indifferent (jugular) blood, and this at *30* and *32* by the second and fourth adrenal blood specimens, respectively. All the bloods were diluted with an equal volume of Ringer's solution. At *35* Ringer was replaced by jugular blood, and this at *36* by jugular blood to which adrenalin had been added to make up a concentration of 1: 2,700,000, the adrenalin blood being then diluted with its own volume of Ringer's solution before application to the segments.[2]

[2] Figures 1 to 13 are tracings from a rabbit intestine segment. All have been reduced to two-thirds. The tracings in figures 1, 2, 8 and 9 were taken with a lighter weight than those in the other figures. Figures 1 to 7 are from bloods of one cat (experiment 1); figures 8 to 13 from bloods of another cat (experiment 2). Figure 14 shows tracings from a rabbit uterus segment, reduced to one-half, with blood from a dog (experiment 3). Figures 15 to 17 are rabbit intestine tracings with bloods from a dog (experiment 5), reduced to two-thirds. In all the tracings time was marked in half-minutes.

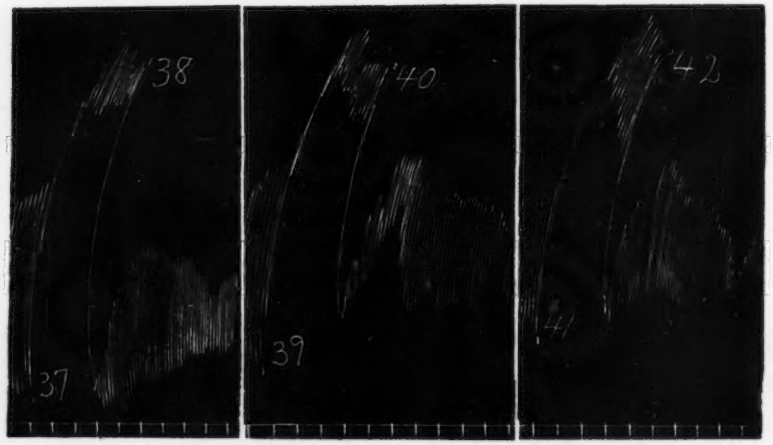

Fig. 2. At *37* and *39* Ringer's solution was replaced by jugular blood diluted with an equal volume of Ringer's solution. At *38* and *40* this was replaced by jugular blood made up with adrenalin to a concentration of 1: 2,000,000 and 1: 3,-400,000, respectively, the adrenalin blood being diluted with an equal volume of Ringer's solution before application to the segment. At *41* Ringer's solution was replaced by jugular blood, and this at *42* by the second adrenal blood specimen, both bloods being diluted with an equal volume of Ringer.

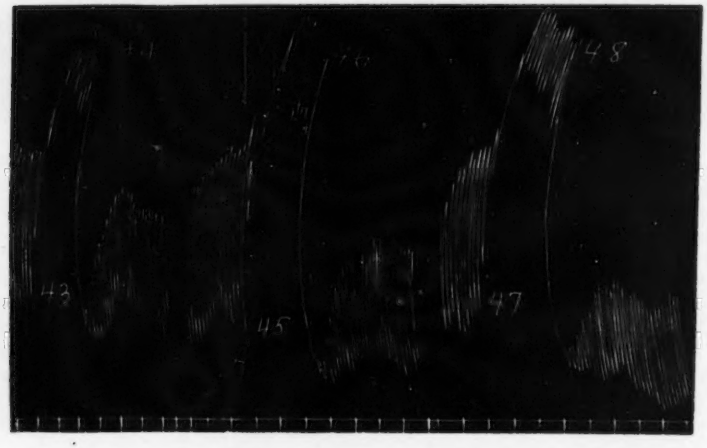

Fig. 3. At *43*, *45* and *47* Ringer's solution was replaced by jugular blood, and this at *44*, *46* and *48* by the first, third and fourth adrenal specimens, respectively. All the bloods were diluted with an equal volume of Ringer's solution before application to the segment.

that the inhibitory effect of the blood on the intestine was due to a substance which caused an increase of tone of the virgin rabbit uterus, that is, to epinephrin.

In figure 2 it is indicated that the second adrenal specimen (observation 42) has a somewhat greater concentration of epinephrin than 1:3,400,000 (observation 40); but decidedly less than 1: 2,000,000 (observation 38), which, however, is not much different from the

Fig. 4. At *49* and *51* Ringer's solution was replaced by jugular blood, and this at *50* and *52* by the second and the fifth adrenal blood specimens, respectively. All the bloods were diluted with an equal volume of Ringer. At *61* Ringer's solution was replaced by jugular blood diluted with its own volume of Ringer, and this at *62* by jugular blood made up with adrenalin to a concentration of 1: 1,350,000, the mixture being then diluted with an equal volume of Ringer's solution before application to the segment.

concentration in the fourth specimen (observation 32, fig. 1). Figure 3 clearly indicates the progressive increase in epinephrin concentration in the first (observation 44), third (observation 46) and fourth specimens (observation 48). Comparison of figure 4 with figure 3 shows that the second specimen (observation 50) does not differ much from the first (observation 44). It must be remembered that the first specimen will contain the epinephrin, if any, liberated by the manip-

ulation incidental to clipping off the upper end of the cava pocket before beginning the collection of blood. On the other hand, a little indifferent cava blood may be included in the pocket when it is clipped off, and this would dilute the first specimen. The result of the tests on the first adrenal specimen shows that in this experiment little epinephrin was liberated by manipulation. The fifth specimen (observation 52, fig. 4) has a much greater concentration of epinephrin than any of the others, and distinctly greater than 1: 1,350,000 (observation 62). No estimate can be formed of the magnitude of the difference between the concentration in the fifth specimen and 1: 1,350,000

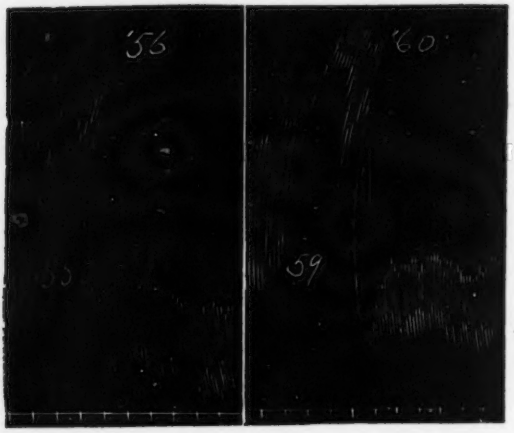

Fig. 5. At *55* and *59* Ringer's solution was replaced by jugular blood diluted with an equal volume of Ringer, and this at *56* and *60* by jugular blood made up with adrenalin to a concentration of 1: 2,000,000 and 1: 2,700,000, respectively; the adrenalin bloods being then diluted with an equal volume of Ringer before application to the segment.

from these two observations, because the inhibitory effect on the intestine segment approaches the maximum effect too nearly. To sharpen the assay greater dilutions of the blood specimen were used later on in the experiment.

Figure 5 indicates that adrenalin in indifferent blood in a concentration of 1: 2,000,000 (observation 56), gives practically the same inhibition as the fourth adrenal blood specimen similarly diluted (observation 48, fig. 3). Observation 60 (fig. 5) shows that a con-

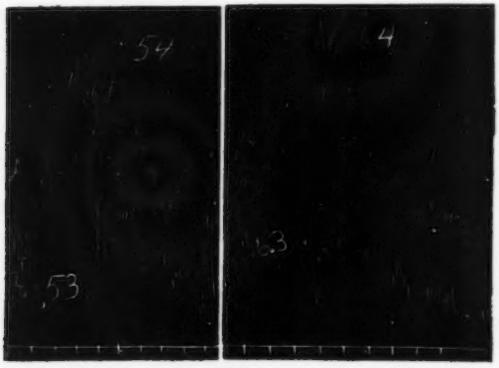

Fig. 6. At *53* Ringer's solution was replaced by jugular blood diluted with its own volume of Ringer and this at *54* by jugular blood made up with adrenalin to a concentration of 1: 3,400,000, the adrenalin blood being then diluted with its own volume of Ringer. At *63* Ringer's solution was replaced by jugular blood and this at *64* by the first adrenal blood sample, both bloods being diluted with an equal volume of Ringer.

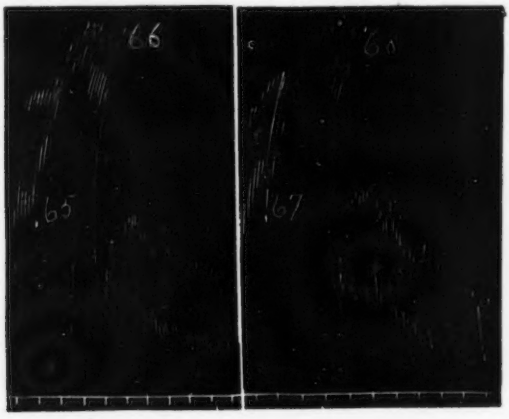

Fig. 7. At *65* and *67* Ringer's solution was replaced by jugular blood diluted with its own volume of Ringer. At *68* this was replaced by the fourth adrenal specimen similarly diluted. At *66* the diluted jugular blood was replaced by a mixture containing one part of the fifth adrenal blood, one part of jugular blood and two parts of Ringer's solution. The ultimate dilution of the epinephrin in the fifth specimen was thus twice as great as in the fourth specimen at observation *68*.

centration of 1:2,700,000 is quite distinctly less than that of the fourth specimen (observation 48, fig. 3), but probably somewhat greater than that of the third specimen (observation 46, fig. 3). In figure 6 it is seen that the first adrenal blood sample (observation 64) has a concentration of epinephrin little different from 1:3,400,000 (observation 54), but probably slightly greater. In figure 7 the fourth specimen was compared with the fifth in greater dilution than in figures 3 and 4. Observation 68 shows the effect of the fifth specimen when the epinephrin in it was diluted twice as much as the epinephrin in the fourth specimen (observation 66). The dilution was made in such a way (see legend of figure) that the concentration of blood was the same in the two observations. It is obvious that the curve in observation 68 falls more sharply after the first abrupt drop than that in observation 66, so that in three minutes it has more nearly approached the base line. There is no doubt, then, that the fifth specimen contains more than twice as much epinephrin as the fourth. It should have three times as great a concentration if the rate of liberation was constant during this period of the experiment, since the rate of blood flow during collection of the fifth sample was only one-third of that during collection of the fourth. It is necessary, however, to point out that as concentrations much greater than 1:1,000,000 are not found in the adrenal vein blood of cats, at least as collected under our experimental conditions and assayed on rabbit segments, the strict inverse ratio of concentration and blood flow is bound to fail for samples collected in the neighborhood of the maximum possible concentration. If we compare the second and fourth specimens, leaving out the first for the reason mentioned, although it certainly does not differ much from the second, we obtain between the concentrations (1:2,000,000 for the fourth, $\pm$ 1:3,400,000 for the second) a ratio of 1:1.7. The ratio between the blood flows is also 1:1.7. Taking the concentration of the third specimen as 1:2,700,000, the ratio between the concentrations of the third and fourth specimens is 1:1.4. The ratio between the blood flows of the fourth and third specimens is also 1:1.4.

Taking the concentration of the second specimen as 1:3,400,000 and that of the third as 1:2,700,000, the ratio between the concentrations is 1:1.3. The ratio between the blood flows of the two specimens is 1:1.2. It would be absurd to refine on such calculations, considering the degree of accuracy possible in epinephrin assays even under the best conditions with such small concentrations as are present in blood. But it is clear enough that in this experiment, for a considerable range of blood flow, the rate of liberation of epinephrin must be assumed to

Experiment 2. Protocol. Cat (pregnant). Weight, 2.975 kgm. Urethane, 4.5 grams. Tracheal and jugular cannulae inserted, and a small sample of jugular blood obtained. Then a short cava pocket was made with ligation of renal arteries and abdominal aorta, and the following specimens of adrenal blood collected.

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION	BLOOD FLOW PER MINUTE
	grams	*minutes*	*grams*
1	5.2	2	2.6
2	9.3	4	2.3
3	11.6	5	2.3
4	10.6	5	2.1
5	11.1	6	1.8
6	5.4	10	0.5

The sixth specimen was collected while additional blood was being obtained from the jugular (40 cc.). Combined weight of adrenals, 0.58 gram.

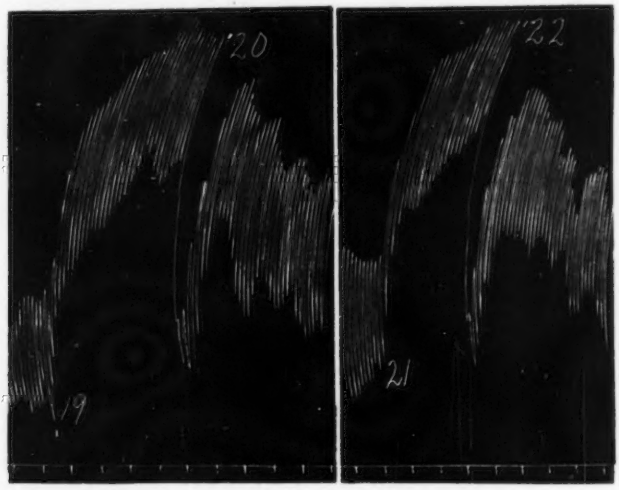

Fig. 8. At *19* Ringer's solution was replaced by indifferent (jugular) blood diluted with an equal volume of Ringer, and this at *20* by the second adrenal blood sample (from experiment 2) similarly diluted. At *21* Ringer's solution was replaced by jugular blood diluted with an equal volume of Ringer, and this at *22* by jugular blood made up with adrenalin to a concentration of 1: 4,000,000, the adrenalin blood being then diluted with its own volume of Ringer before application to the segment. The weight used was less than in the other figures from experiment 2 except figure 9.

have remained approximately constant. It necessarily follows that the concentration of epinephrin in the blood of the adrenal veins must have varied inversely as the blood flow through the glands.

The same thing is demonstrated fully as well in experiment 2. The initial concentration in this experiment being smaller than in the previous one, the rule of the inverse ratio applies even to the specimen collected with the slowest flow.

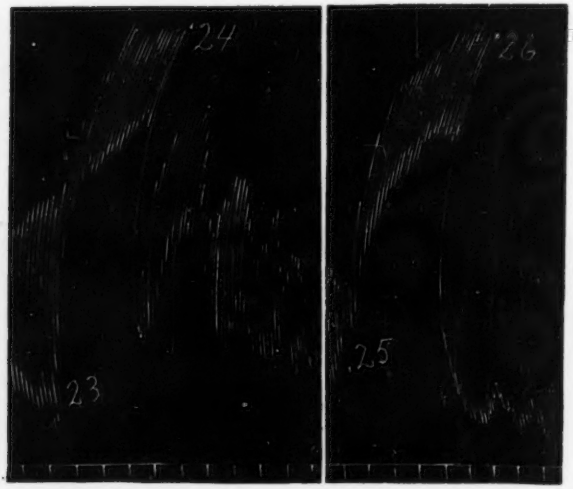

Fig. 9. At *23* and *25* Ringer's solution was replaced by jugular blood diluted with its own volume of Ringer, and this at *24* and *26* by jugular blood made up with adrenalin to concentrations of 1: 2,700,000 and 1: 1,350,000 respectively; the adrenalin bloods being then diluted with an equal volume of Ringer's solution before application to the segment. The weight used was less than in the other figures from experiment 2 except figure 8.

The bloods from experiment 2 were tested on the same intestine segment as those from experiment 1. Figure 8 shows that the second adrenal blood specimen from experiment 2 (observation 20) produces an effect on the intestine nearly the same as that produced by indifferent (jugular) blood containing adrenalin in the concentration of 1: 4,000,000. The epinephrin concentration in this specimen was shown to be decidedly less than 1: 2,700,000, and very much less than 1: 1,350,000 (fig. 9, observations 24 and 26). These curves are reproduced to prove that

the sensitiveness of the segment to different concentrations of adrenalin in blood was sufficient for the purposes of the assay. Figures 10 and 11 display the effects of all the adrenal blood specimens from experiment 2 on the intestine segment. The second and third specimens (observations 72 and 74, fig. 10) produced practically the same inhibition, and it will be seen from the protocol that during their collection the blood flow remained unchanged.

These tracings cannot be quantitatively compared with those reproduced in figures 8 and 9, because the weight attached to the lever

Fig. 10. At *69*, *71* and *73* Ringer's solution was replaced by jugular blood and this at *70*, *72* and *74*, respectively, by the first, second and third adrenal blood specimens (experiment 2). All the bloods were diluted with an equal volume of Ringer's solution.

had been increased after observation 42 (fig. 2). Also, as already mentioned, it is a rule in this method of assaying only to compare observations not very far apart in the series. The somewhat greater inhibitory effect caused by the first specimen (observation 70, fig. 10) than by the second or third is doubtless due to some slight "manipulative" liberation of epinephrin while the pocket was being closed off. Observation 80 (fig. 11) indicates a slightly greater concentration for the fourth adrenal sample than for the third (observation 74, fig. 10), and the rate of blood flow during collection of the fourth specimen was slightly less than during collection of the third. Observation 82 (fig. 11) suggests that the epinephrin concentration in the fifth specimen is

somewhat greater than in the fourth. In particular, the transient rise following the first abrupt drop is less than in observation 80. The blood flow during collection of the fifth specimen was somewhat less than during collection of the fourth. The inhibition produced by the sixth adrenal blood sample (observation 84, fig. 11) obviously corresponds to a very much greater epinephrin concentration than that of any of the other specimens, and the protocol shows that the blood

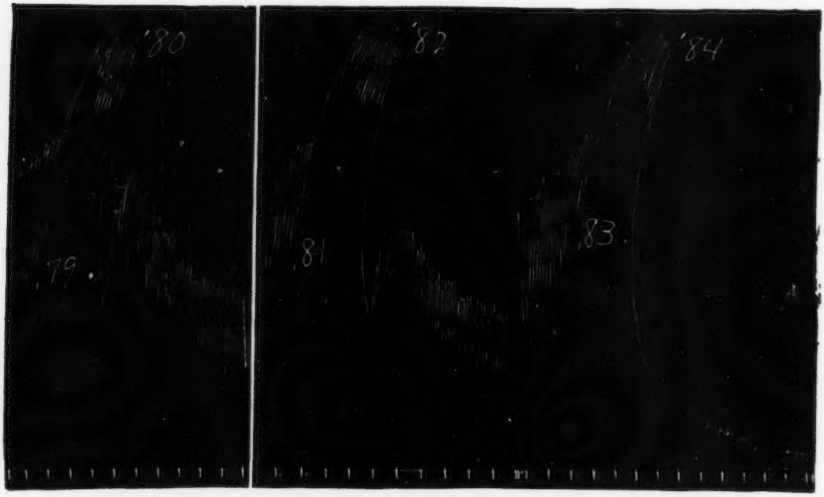

Fig. 11. At *79*, *81* and *83* Ringer's solution was replaced by jugular blood, and this at *80*, *82* and *84*, respectively, by the fourth, fifth and sixth adrenal blood specimens (experiment 2). All the bloods were diluted with one volume of Ringer's solution. The drum was lowered in the interval between figures 10 and 11, so as to obtain space for the drop expected in observation *84*. The greater distance of the curves from the base line accordingly does not indicate that the tone of the segment beating in Ringer's solution had increased.

flow during collection of the sixth specimen was by far the smallest of all. The observations illustrated in figures 10 and 11 are sufficient to show qualitatively an inverse relation between the rate of blood flow and the epinephrin concentration. Figures 12 and 13 complete the quantitative proof. Observation 86 (fig. 12) indicates that 1: 2,700,000 is a little greater than the concentration in the fifth specimen (observation 82, fig. 11). The initial drop in observation 86 is somewhat greater,

and the subsequent transient recovery of tone decidedly less than in observation 82. It was previously determined that the concentration in the second specimen of experiment 2 was approximately 1: 4,000,000. If the concentration in the fifth specimen be taken even as 1: 2,700,000, and it is less than this, the ratio between the concentrations of the second and fifth specimen is 1: 1.5, whereas the ratio between the blood flows of the fifth and second specimens is 1: 1.3. If the concentration of the fifth specimen be taken as 1: 3,000,000, the ratio between the

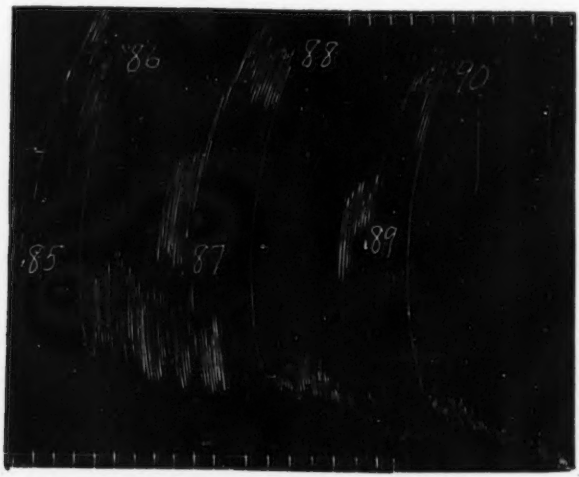

Fig. 12. At *85*, *87* and *89* Ringer's solution was replaced by jugular blood diluted with an equal volume of Ringer, and this at *86*, *88* and *90* by jugular blood made up with adrenalin to concentrations of 1: 2,700,000, 1: 1,350,000 and 1: 700,000 respectively, the adrenalin bloods being then diluted with an equal volume of Ringer before application to the segment.

concentrations will also be 1: 1.3. Comparing observation 84 (fig. 11) with observations 88 and 90 (fig. 12). it is clear that the concentration in the sixth specimen is superior to 1: 1,350,000, and not very different from 1: 700,000. The response of the intestinal segment to such high concentrations of epinephrin is, however, too near the maximum response for the greatest accuracy of which the method is capable. Accordingly observations were made with a greater degree of dilution.

In figure 13 it is shown that the concentration of epinephrin in the sixth specimen (observation 92) is somewhat less than 1: 700,000 (ob-

servation 94), and decidedly greater than 1:950,000 (observations 98 and 100). If the concentration in the sixth specimen be taken as 1:800,000 and that in the fifth specimen as 1:3,000,000, the ratio between the concentrations of the fifth and sixth samples comes out 1:3.7. Taking the concentration in the fifth specimen as 1:2,700,000, the ratio would be 1:3.4. The ratio between the blood flows for the sixth and fifth specimens is 1:3.6. In this experiment, with a range of blood flow from 2.3 grams per minute to 0.5 gram per minute, the inverse ratio between blood flow and epinephrin concentration in the adrenal blood was maintained; in other words, the rate of liberation of epi-

Fig. 13. At *91* Ringer's solution was replaced by jugular blood diluted with its own volume of Ringer. At *92* this was replaced by a mixture containing one part of the sixth adrenal blood, (from experiment 2) one part of jugular blood and two parts of Ringer's solution. At *93* Ringer's solution was replaced by jugular blood diluted with its own volume of Ringer, and this at *94* by a mixture containing ing one part of jugular blood made up with adrenalin to a concentration of 1:700,000, one part of jugular blood and two parts of Ringer. At *97* Ringer's solution was replaced by jugular blood, diluted with two volumes of Ringer, and this at *98* by the sixth adrenal specimen (from experiment 2) similarly diluted. At *99* Ringer's solution was replaced by jugular blood diluted with two volumes of Ringer, and this at *100* by jugular blood made up with adrenalin to a concentration of 1:950,000, the adrenalin blood being then diluted with two volumes of Ringer before application to the segment.

nephrin remained constant for the whole of this experimental period. It must be assumed that in this animal even when the very high concentration of 1:800,000 had been reached in the blood, the glands were still able to pass into the blood the full amount of epinephrin delivered per unit of time with greater blood flows. Since, however, 1:800,000 represents a concentration scarcely ever surpassed in adrenal blood, collected and assayed in the manner described, it may be concluded with confidence that had collection of the adrenal blood been continued with still smaller rates of flow, the inverse proportionality would of necessity have disappeared and the amount of epinephrin liberated per minute into the blood would have shown a progressive diminution.

In general it may be said that the approximate constancy of the rate of epinephrin liberation under the experimental conditions can be demonstrated throughout the greatest range of blood flow when the initial concentration is low, or what usually although not invariably comes to the same thing, when the initial blood flow is high. When the concentration in the first samples is already high, the rate of liberation will soon appear to diminish as the rate of blood flow falls below the point corresponding to the maximum possible concentration. A few more results illustrating this rule may be quoted in summarized form.

In a cat weighing 2.035 kgm., the protocol of which has been published elsewhere (3), the second adrenal blood specimen, with a flow of 2.2 grams per minute, had a concentration of about 1:2,500,000 of epinephrin; and the third adrenal specimen, with a flow of 1.3 gram per minute, a concentration of about 1:1,500,000. The ratio of the concentrations of the second and third specimens is 1:1.7, and the ratio of the blood flows of the third and second specimens is also 1:1.7. The output of epinephrin was therefore unchanged during collection of the two samples (0.0009 mgm. per minute). This is a relatively large output as assayed in drawn blood on rabbit segments. In the fifth adrenal specimen, the output calculated from the epinephrin assay was much less, 0.0004 mgm. per minute. But this was inevitable, since the blood flow was only 0.4 gram per minute, and the concentration which was already approaching the maximum limit during collection of the third specimen, could not increase in proportion to the decrease of blood flow, although it reached the high value of 1:1,000,000.

In another cat, weighing 2.66 kgm. (2), the concentration in the second adrenal sample was 1:4,500,000, and in the fifth, 1:2,300,000. The blood flows were 2.1 grams and 1.0 gram per minute, respectively. The ratio between the concentrations of the second and fifth specimens

is 1:2, and that between the blood flows of the fifth and second, 1:2.1. The output of epinephrin per minute was therefore practically the same for the two samples, about 0.00045 mgm. per minute.

In another cat, weighing 3.16 kgm., the concentration in the second adrenal specimen was 1:1,700,000, with a blood flow of 1.30 gram per minute, and in the third specimen, 1:1,000,000, with a flow of 0.73 gram per minute. The rate of liberation of epinephrin was relatively large in this animal, and therefore the maximum concentration was reached with a greater blood flow than would have been the case had the output been smaller. The ratio between the concentrations in the two specimens is 1:1.7, and the ratio between the blood flows, 1:1.8. The output of epinephrin per minute was practically the same for the two samples, about 0.00075 mgm.

In a cat weighing 2.435 kgm. the concentration of epinephrin was found the same, namely 1:2,000,000, in the second, third and fourth adrenal blood specimens, and the blood flows were also equal (1.3 gram per minute). The output of epinephrin per minute was 0.0006 mgm.

In a cat weighing 2.31 kgm., the unusually low concentration of 1:13,000,000 of epinephrin was found in the second adrenal blood specimen, associated with the unusually high blood flow of 5.0 gram per minute (4). In the eighth adrenal specimen, after section of the cord in the cervical region, the concentration mounted to something less than 1:800,000, as great a concentration of epinephrin as we have ever seen in the drawn adrenal blood of a cat. The blood flow during collection of the sample was only 0.3 gram per minute. The concentration in the eighth specimen was accordingly about sixteen times as great as in the second, and the rate of blood flow during collection of the eighth specimen was sixteen times smaller than during collection of the second. The output of epinephrin therefore remained unaltered, namely, 0.0004 mgm. per minute.

In dogs the available data are less extensive but similar results have been obtained. In an experiment on a dog weighing 14.9 kgm., the protocol of which has been published in another connection (4), the concentration of epinephrin in the fifth adrenal blood specimen was about 1:3,000,000; and in the eighth specimen, about 1:1,100,000. The blood flows were 5.7 cc. and 1.8 cc. per minute. The rate of liberation of epinephrin per minute was therefore practically the same in the two samples, notwithstanding the great difference in the blood flow. No doubt the correspondence would have been still more exact had the

concentration in the final sample not been approaching so near the maximum.

In some of the dogs the attempt was made to vary the blood flow through the adrenals by causing incomplete inhibition of the heart by vagus stimulation. However, the effect, was not very decided. The rule abundantly illustrated in the experiments on cats, that a specimen of adrenal blood collected with a slower flow has a greater concentration of epinephrin than one collected in the same experiment with a faster flow, was verified in every case in the observations on dogs. So many intestine tracings having been already reproduced, it will be sufficient to give a sample of uterus tracings (fig. 14, experiment 3) illustrating this point.

Experiment 3. Protocol. Dog (female). Weight, 6.7 kgm. Ether. Cava pocket prepared with cannula in right iliac vein. One vagus prepared for stimulation in the neck. The following samples of blood were then collected:

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	cc.	*minutes*	*seconds*	*cc.*	
1	28.0	1	45	16.0	
2	31.0	1	50	16.9	
3	42.0	2	20	18.2	Vagus stimulation
4	30.5	2	45	11.9	Vagus stimulation
5	20.0	2	10	9.2	
6	19.5	3		6.5	Vagus stimulation
7	20.0	4	45	4.2	
8	18.0	7		2.6	
9	8.7	6	30	1.3	

Blood was now collected from the carotid artery and used as the indifferent blood in testing with rabbit intestine and uterus segments. As usual, all bloods were well shaken up with air before being applied to the segments. The combined weight of the adrenals was 0.65 gram.

From the amount of the earlier blood flows, it seems possible that some leakage from other sources than the adrenal veins may have taken place into the pocket, although careful search at autopsy failed to reveal any untied small vein. It must be remembered, however, that with so many arteries clamped the arterial blood pressure is very high at first, and a large blood flow through the adrenals would therefore be expected. In any case, the relative concentration of epinephrin in the successive adrenal samples would probably not be affected, even if a small vein had been overlooked.

The specimens of uterus tracings reproduced in figure 14 suggest that the second adrenal sample (observation 81) has a slightly higher concentration of epinephrin than the third sample (observation 75), corresponding to the slightly greater flow during collection of the latter, notwithstanding the vagus stimulation. The fourth sample (observation 76) has a distinctly greater tone-increasing effect than the third; the fifth sample (observation 77) has a greater effect than the fourth, the sixth (observation 78) a greater effect than the fifth, and the seventh (observation 79) a greater effect than the sixth. The increase of tone produced by the seventh sample was shown to be maxi-

Fig. 14. Uterus tracings. Dog's blood (experiment 3). At *75* Ringer's solution was replaced by the third adrenal blood specimen; at *76* by the fourth; at *77* by the fifth; at *78* by the sixth; at *79* by the seventh; at *81* by the second adrenal sample.

mal for the segment, and that is the reason for the relatively small additional increase caused by the seventh specimen. The eighth and ninth samples naturally produced no further increase of tone. But when the specimens were appropriately diluted a markedly greater effect was caused by the seventh than by the sixth, by the eighth than by the seventh, and by the ninth than by the eighth. Corresponding results were obtained on the intestine segments.

In another dog (experiment 4), a similar experiment was performed and with a similar result.

The epinephrin content of the second adrenal specimen was assayed on a rabbit intestine segment at 1: 6,000,000, corresponding to an output of 0.001 mgm. of epinephrin per minute for the animal (0.00015 mgm. per kilogram of body weight per minute). The epinephrin con-

tent of the sixth adrenal specimen was assayed at somewhat more than 1:3,000,000, corresponding to an output of more than 0.0009 mgm. per minute for the animal, practically the same as for the second specimen. The concentrations in the two specimens were therefore approximately in the inverse ratio of the blood flows. Several additional experiments were made on dogs, of which only one more will be cited.

Experiment 4. Protocol. Dog (female in early pregnancy). Weight, 7.7 kgm. Ether. Cava pocket made. Renal and iliac arteries and left iliac vein tied. Cannula inserted into right iliac vein. Left vagus cut in the neck and its peripheral end prepared for stimulation. The following specimens of adrenal blood were then collected.

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	cc.	*minutes*	*seconds*	*cc.*	
1	13.2	1	40	8.0	
2	13.0	2		6.5	Vagus stimulation
3	12.5	2	30	5.0	Vagus stimulation
4	16.6	4	28	3.7	
5	12.6	4	29	2.8	
6	7.0	2	30	2.8	Vagus stimulation

A sample of jugular vein blood (17 cc.) was drawn before collection was begun from the cava pocket. After collection of the adrenal samples, 120 cc. of blood was obtained from the carotid artery. The combined weight of the adrenals was 0.78 gram.

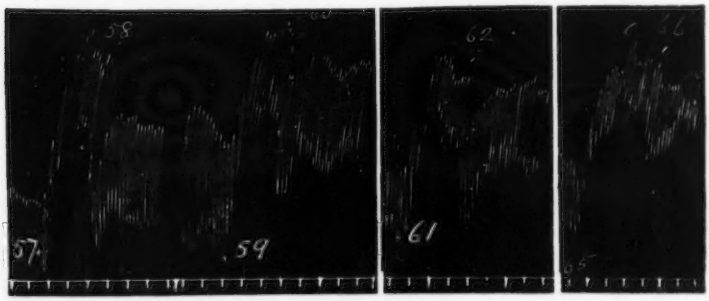

Fig. 15. Intestine tracings. Dog's blood from experiment 5. At *57*, *59*, *61* and *65* Ringer's solution was replaced by indifferent (arterial) blood, and this at *58*, *60*, *62* and *66*, by the tenth, seventh, eighth and fourth adrenal blood specimens respectively. All the bloods were diluted with three volumes of Ringer's solution.

Experiment 5. Dog (male). Weight, 11.3 kgm. Ether. A cava pocket was formed without tying off the intestinal arteries. A blood pressure tracing was taken from the carotid during collection of the adrenal samples. During collection of some of the samples the vagus was stimulated in the neck.

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	BLOOD PRESSURE	REMARKS
	cc.	*minutes*	*seconds*	*cc.*	*mm. of Hg.*	
1	21.4	1	28	14.6	96	
2	20.4	1	25	12.7	92	Vagus stimulation
3	21.9	2	21	9.3	92	
4	16.6	2	15	7.4	82	Vagus stimluation
5	21.9	2	50	7.7	82	Vagus stimulation*
6	17.7	2	30	7.0	82–70	Vagus stimulation*
7	17.6	3		5.8	65	
8	21.9	3	36	6.0	60	
9	21.7	3	55	5.5	60 falling	
10	22.9	6	15	3.6	falling	
11	12.4	4	33	2.7	28–22	

* The vagus was stimulated during part of the time of collection of these specimens.

Blood was drawn from the jugular vein before collection of the adrenal samples to serve as indifferent blood in the intestine and uterus tests. Combined weight of adrenals, 1.30 gram. Seventy-five cubic centimeters of blood was obtained from the carotid at the end of the experiment. Specific gravity of fifth adrenal specimen, 1.071. Specific gravity of the carotid blood at the end of the experiment, 1.062.

Numerous tracings were taken to compare the effects of the various adrenal blood samples on intestine and uterus segments. A few are reproduced in figures 15 to 17. In figure 15 it is seen that the tenth adrenal specimen (observation 58) inhibits the intestine segment much more powerfully than either the seventh (observation 60) or the eighth (observation 62), and that the seventh and eighth specimens cause a distinctly greater inhibition than the fourth (observation 66). Reference to the protocol will show that the blood flow during collection of the tenth sample was twice as great as during collection of the fourth. When the seventh and eighth specimens were being obtained the rate of blood flow was intermediate in amount. In figure 16, the effects of the fourth (observation 64) and eleventh (observation 68) adrenal blood specimens are compared. A much greater inhibition was produced by the eleventh than by the fourth specimen, which was collected with a blood flow nearly three times as great as during collection of the eleventh sample.

A point of some technical importance may be referred to here. Observations 63 and 64 were made with the same blood sample as observations 65 and 66 (fig. 15), in the same dilution, and very near each other in the series. Yet the curves are not suitable for comparison, since the increase of tone produced at 63 is considerably greater than that at 65. Sometimes a segment rather suddenly changes in its sensitiveness to the tone-increasing action of blood, and we have observed that when the augmentation of tone produced by a given blood or serum is increased, the inhibition caused by a given concentration of epinephrin in blood or serum is also increased. Thus the absolute amount of the drop in the curve at 64 is greater than at 66. This phenomenon, which does not occur very frequently, does not interfere at all with the estimation of epinephrin in blood samples, provided the rule is obeyed that only curves which can be properly compared are used for comparison. After washing the segment more thoroughly with Ringer's solution and allowing a longer interval before application of the blood, it began again to give curves like 65 and 66, which are comparable with the others reproduced in figure 15.

In figure 17, are shown a few of the tracings taken with the same bloods, but on a segment from another rabbit. Again, of course, observations in this series cannot be compared with observations on the same bloods in the series on the other segment. Thus, observations 16 (fig. 17) and 68 (fig. 16) were both obtained with the eleventh adrenal blood specimen and observations 31 (fig. 17), 64 (fig. 16) and 66 (fig. 15) with the fourth specimen. Observations 18 and 23 (fig. 17) were made with the same blood (the ninth adrenal sample) and on the same intestinal segment, but the segment was differently weighted, and they cannot be compared for quantitative purposes. It is sometimes advantageous after making an epinephrin assay on a segment to change the weight or to change the segment and to repeat the assay. Each curve in the second series will differ from the corresponding curve in the first. If a new segment has been taken, there may be no resemblance between the corresponding curves in the two series, but this will not interfere in the least with the comparison of the

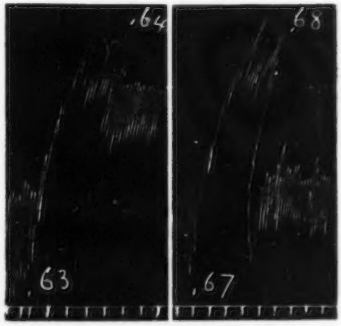

Fig. 16. Intestine tracings. Bloods from the same dog used for figure 15. At *63* and *67* Ringer's solution was replaced by indifferent (arterial) blood and this at *64* and *68* by the fourth and eleventh adrenal blood specimens respectively. The bloods were diluted with three volumes Ringer's solution.

curves in one and the same series, and the relative position of the various blood samples as regards their content of epinephrin will be identical for the two series.

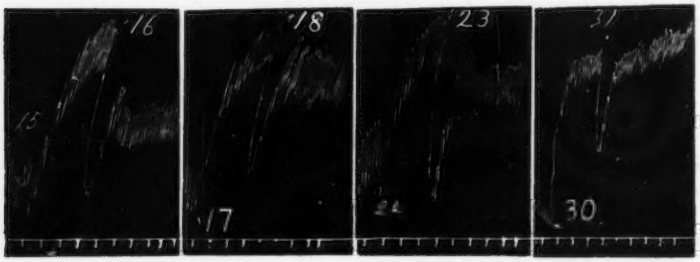

Fig. 17. Intestine tracings. Bloods from the same dog used for figures 15 and 16. The intestine segment was from another rabbit. At *15* and *17* Ringer's solution was replaced by indifferent (jugular) blood, and this at *16* and *18* respectively by the eleventh and ninth adrenal blood specimens. After *18* the weight was increased. At *22* and *30* Ringer's solution was replaced by jugular blood, and this at *23* and *31* by the ninth and fourth adrenal blood specimens, respectively. All the bloods were diluted with three volumes of Ringer's solution.

SUMMARY

It is shown that within the limits of error of the method used for the assay (rabbit intestine and uterus segments) the concentration of epinephrin in the adrenal vein blood collected from a cava pocket varies in different samples from the same animal inversely as the rate of blood flow through the glands during the period of collection, the rate of output of epinephrin per minute being constant under the experimental conditions for a considerable range of blood flow.

The rule fails, of course, when the rate of blood flow is diminished below the value at which the concentration of epinephrin has reached the possible maximum. For this reason determinations of the rate of spontaneous liberation of epinephrin by assays on drawn adrenal vein blood should be made on samples obtained with a rate of flow well above this limiting value.

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THE EFFECT OF VARYING THE CALCIUM AND POTASSIUM CONTENT OF PERFUSION FLUIDS ON VAGUS INHIBITION

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Busquet and Pachon (1) in studying the phenomenon of inhibition came to the conclusion that saline solutions which interfered with the functioning of the vagus when perfused through the heart would lose this faculty by the addition of calcium. Thus they attribute the activity of calcium upon the cardio-inhibitory mechanism to the presence of free ions in the muscle cell. Hagan and Ormond (2) agree with the conclusions of Busquet and Pachon that calcium is a necessary factor in inhibition. These latter writers state that if vagus control be depressed its activity can be restored by the addition of a small amount of calcium to the perfused solutions. However, they think that it is necessary to the proper functioning of the nerve apparatus (not the muscle cell as Busquet states). Just what part of the nervous mechanism is acted upon is not yet evident. They suggest that it may be the nerve fibers, the nerve terminals or the receptive substance. These conclusions were reached from experiments testing the individual effect of different salines on vagus control. In duplicating these experiments in an attempt to throw further light upon the theory of inhibition the results obtained were so totally different that it seemed wise to present these negative results, and the problem at issue re-resolved itself into an investigation of the effect of various saline solutions on vagus inhibition rather than an inquiry into the nature of inhibition itself.

METHOD

The animals used were terrapin. Proper irrigation of the heart with the different salines was effected by a perfusion apparatus origin-

ally devised by Sutherland Simpson (3). Difficulty was experienced in finding the most advantageous place to introduce the cannula in order not to interfere with the nerve supply. It was found that the most satisfactory position was as far away from the heart as possible, just where the vena cava passes through the liver, approximately at the junction of the sinus and this vein.

In order to obtain the effect of each solution alone on the heart, another cannula was inserted into the aorta so that the outflowing solution could not bathe the heart. The right vagus was exposed and stimulated at intervals with a tetanizing current. The threshold stimulus which would give complete inhibition was determined in each case. Records of the action of the heart were recorded on a slow drum.

The mode of procedure was as follows: Ringer's solution was perfused first through the heart for several minutes, always determining the threshold stimulus for inhibition. Then perfusion of this solution was stopped and the first solution to be tested was allowed to irrigate the heart and the vagus was again stimulated (five to ten seconds) with the same threshold strength as that for Ringer's solution.

If no inhibition occurred the strength of the current was gradually increased until the new threshold value was obtained. Between the irrigation of each of the different fluids, Ringer's was perfused in order to keep the contraction as normal as possible. The heart was moistened frequently to prevent it from drying. The composition of the various solutions was as follows:

SOLUTION	NaCl	KCl	$CaCl_2$
	per cent	*per cent*	*per cent*
I	0.7		0.003125
II	0.7	0.0075	0.003125
III	0.7	0.015	0.003125
IV	0.7	0.0075	0.00625
V	0.7	0.0075	

The results are as follows:

Results

SOLUTION	NUMBER OF ANIMAL*	STRENGTH OF CURRENT SECONDARY AT†	REACTION
NaCl 0.7 per cent, $CaCl_2$ 0.003125 per cent	5	11 Threshold value for	Inhibition
NaCl 0.7 per cent, $CaCl_2$ 0.003125 per cent	3	8 Threshold value for	Inhibition
NaCl 0.7 per cent, KCl 0.0075 per cent, $CaCl_2$ 0.003125 per cent	3	10 Threshold value for	Inhibition
NaCl 0.7 per cent, KCl 0.0075 per cent, $CaCl_2$ 0.003125 per cent	13	5 Threshold value for	Inhibition
NaCl 0.7 per cent, KCl 0.015 per cent, $CaCl_2$ 0.003125 per cent	4	11 Threshold value for	Inhibition
NaCl 0.7 per cent, KCl 0.015 per cent $CaCl_2$ 0.003125 per cent	10	4½ Threshold value for	Inhibition
NaCl 0.7 per cent, KCl 0.015 per cent, $CaCl_2$ 0.003125 per cent	11	6 Threshold value for	Inhibition
NaCl 0.7 per cent, KCl 0.0075 per cent, $CaCl_2$ 0.00625 per cent	4	11½ Threshold value for	Inhibition
NaCl 0.7 per cent, KCl 0.0075 per cent, $CaCl_2$ 0.00625 per cent	13	5 Threshold value for	Inhibition
Ringer's NaCl 0.7 per cent, KCl 0.03 per cent, $CaCl_2$ 0.026 per cent	Majority of animals used	12 Threshold value for	Inhibition
NaCl 0.7 per cent, KCl 0.0075 per cent	3	12 Threshold value for	Slight slowing rate
NaCl 0.7 per cent KCl 0.0075 per cent	3	11 Threshold value for	Inhibition
NaCl 0.7 per cent KCl 0.0075 per cent	13	6 Threshold value for	Inhibition
NaCl 0.7 per cent KCl 0.0075 per cent	2	4 Threshold value for	Inhibition
NaCl 0.7 per cent KCl 0.0075 per cent	1	8 Threshold value for	Inhibition

* This refers to the fact that the animals used were numbered respectively 1, 2, etc.

† Harvard apparatus inductorium.

STATEMENT OF RESULTS

As can be seen from the above table, inhibition was obtained in all cases and the results therefore differ considerably from those of Hagan and Ormond as they were unable to obtain inhibition when solutions I, II and V were perfused through the heart. They secured only partial inhibition with solution III. The two results which do coincide were the reactions obtained by Ringer's, and solution IV (NaCl 0.7 per cent, KCl 0.0075 per cent, $CaCl_2$ 0.00625 per cent).

DISCUSSION OF RESULTS

The above disparities cannot as yet be satisfactorily explained. As these experiments extended over a period of two years, no seasonal variation in the animals could be considered as the animals were used during all seasons of the year. The only noticeable exception was that the threshold value for inhibition was much higher for all the animals used during the winter, 1916, and spring, 1917, than for the other animals. Two different kinds of terrapin were employed (Pseudimys Rugosa, the slider terrapin and Chrysemys picta.) In order to obtain only the effect of the liquid perfused and not that of the preceding one, great care was given to the length of perfusion. The solution to be tested was allowed to irrigate the heart for eight to ten minutes in some cases even longer until the heart gave signs of abnormal contractions. In every case the effect of the individual solutions manifested its own reaction on the contraction of the heart. Even with continued irrigation as stated above, vagus control was still present. Thus it could not be a question of the individual salines not exerting their own effect. It may be mentioned that it was impossible to accurately compare these results with those obtained by Hagan and Ormond as the exact strength of the calcium used by them could not be determined. They did not state whether or not water of crystallization was taken into consideration in the calculation of the percentage strength of this substance in solution. On account of this fact the solutions used in these experiments were made up in two ways, with and without taking into account the water of crystallization. The results showed, however, that both series of solutions gave similar results. A stock solution of 0.5 per cent calcium chloride (1 gram of dried calcium chloride crystals made up to 200 cc. with water) was used in preparing the various solutions. In trying to explain why solutions I, II and V did not interfere with vagus control it may be

stated that when these fluids were perfused through the heart and failed to interfere with vagus control, there was no way of determining that the heart had lost some of the calcium which the heart muscle liberated during its previous activity. Even though these solutions were deficient in their calcium content, it may be that enough free calcium remained in the heart to exert its usual control on the action of the vagus. It is also possible the length of perfusion performed by Hagan and Ormond was longer than in these experiments, and thus all free calcium may have been removed, and when the solutions weak in calcium were perfused no free calcium was present to exert its effect upon vagus control. These investigators, however, do not state the duration of perfusion.

The fact that, as the result of our perfusion, a change in rhythm manifested itself in the heart muscle would indicate that an alteration in the salt equilibrium of the cell *had* occurred.

I desire to express my appreciation of the suggestions given by Prof. Holmes C. Jackson during the course of this work.

CONCLUSION

Calcium free solutions or solutions with a very small percentage of calcium do not interfere with vagus activity contrary to results previously reported.

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THE ACTION OF ADRENALIN ON THE BLOOD

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It has been discovered that the addition of adrenalin to venous blood causes a broadening and intensification of the absorption bands of oxyhaemoglobin, due presumably to an increase in the content of oxyhaemoglobin. This same action of adrenalin is also made evident in the blood of the adrenal vein. It is inferred that adrenalin is for this reason an important factor in the oxygenation of the blood in the lungs, increasing there the degree and velocity of the oxidation of haemoglobin.

INTRODUCTION

The significance of the position of the adrenal glands has not hitherto been generally recognized. The secretion from these glands is known to be rapidly destroyed in the blood, adrenalin being very easily oxidized. The principal action of the drug must then in the very nature of things be upon those organs which it reaches in the greatest concentration. These organs are the heart and lungs. Adrenalin is poured into the blood of the vena cava just before that blood reaches the heart. It does not go into the portal circulation, as it presumably would were its chief function to act upon the liver; but it goes direct to the right heart and lungs. Indeed it is very doubtful whether under normal conditions any but very small amounts ever get by these two organs. It is well known that it does stand in a very special relation to both the heart and lungs. In the former it strengthens particularly the right heart, a fact which is the justification for its use for pneumonia and other cases in which this side of the heart is particularly overloaded; and its action on the lungs (1) dilating the bronchioles is also well recognized. That it may also and does particularly affect the oxygen exchange in that organ has not hitherto been appreciated, but that such is the case is clearly indicated by the experiments which follow.

METHODS

The experiments fall into two groups, one in which the spectrum of the blood from the adrenal vein was compared with that of venous and arterial blood from elsewhere in the body, and a second group in which the absorption spectra of the same sample of venous blood, with and without the addition of adrenalin, were studied.

The first obstacle encountered in these studies was that of obtaining blood in layers sufficiently thin to allow the spectra to be photographed without too long an exposure. Because of the decomposition of the blood containing adrenalin it was imperative that the exposure time should not exceed at the most one and a quarter hours. For the solution of this first difficulty the author is indebted to the suggestion of Mr. Siegfried Maurer, of reducing the inside dimensions of the ordinary U-shaped spectroscope absorption cell by the insertion of a piece of glass, ground to fit closely, so as to obtain a layer of the thickness of 1.2 mm. Even a layer of this thickness, however, necessitated reducing the density of the blood by dilution. As dilution fluid, various solutions of sodium chloride ranging in concentration from 0.6 to 0.9 per cent Tyrode's solution with and without dextrose and dog serum were tried. Dilution media which caused the corpuscles to settle out of the fluid had to be avoided. In the later experiments with human blood only 0.85 per cent sodium chloride was used.

It was found advisable to have the percentage oxygen saturation of the diluted blood to be examined not greater than that occurring normally in the circulating blood. To secure this condition, the dilution fluid was rendered practically oxygen-free by means of a vacuum pump, sealed and exposed to the air again only immediately before being used. Subsequent procedure depended upon whether the experiments belonged to the first or second groups previously mentioned. The technique employed in the second group being the simpler, will be outlined first. In these experiments, in which the effect of adrenalin on a sample of blood was to be tested, venous blood was withdrawn from the animal, as rapidly as possible made up to the required dilution with the de-oxygenated dilution medium chosen and divided into two parts, to one of which adrenalin varying in different experiments from 1 in 1,000,000, to saturation was added. Both specimens were then poured directly into the absorption cells, which were immediately sealed and their spectra secured at once. The dilutions employed were such as to give concentrations of blood of 10 per cent, 6.6 per cent and

5 per cent. With an exposure of one and a quarter hours, the first of these gave the most satisfactory results. Although clotting of blood at these concentrations usually does not occur, in some cases hirudin was added, but the presence of this substance revealed no apparent difference in the spectra obtained.

To avoid any variation which might arise from possible varying composition of the adrenalin used, only one sample of this substance was used throughout the series of experiments. Since Tribe (2) has reported that the presence of chloretone in adrenalin chloride solution may be a possible factor in modifying the action produced by adrenalin, only the free base was used. This was a Parke-Davis preparation made from the gland, and in appearance was a fine yellowish powder of which the activity had been previously ascertained by physiological tests and by increase in alkalinity produced when it was added in definite quantities to serum, of which the normal reaction was known.

When the experiments belonged to the first group, in which samples of blood from different parts of the body were to be compared for their oxyhaemoglobin content, each sample was withdrawn separately, quickly diluted in one of the proportions mentioned above, poured into an absorption-spectroscope cell, sealed with a glass top and wax so as to exclude completely any further contamination with the oxygen of the air. Its spectrum was then obtained without further delay. In this series the dilution medium for each sample of blood to be studied was placed in a separate flask and made nearly oxygen-free, thus giving as far as possible a minimal contact with the air during the process of dilution. Further, the time of contact of the dilution media with the air was kept as nearly as possible the same in every case so that the variations due to differences in oxygen content were reduced to a minimum. Because of decomposition changes to be discussed later, which occur in blood containing adrenalin, the spectrum of blood from the adrenal vein or from blood to which adrenalin was added was invariably obtained first in a series and as soon after withdrawal as possible. Blood from other parts of the body when sealed in the spectroscopic absorption cells and kept in a cool place showed no appreciable variation in the spectra obtained within forty-eight hours. In blood kept for a longer time than this, reduction of the haemoglobin not infrequently occurs.

The spectra were obtained by means of a Michelson concave grating of six feet radius used in the first order. The source of illumination in all except a few cases was a Nernst glower whose image was formed on

the spectroscope slit by means of a suitably placed lens. In some of the earlier experiments a carbon arc giving a continuous spectrum was employed as a source of light. Although the greater intensity produced by this method of illumination was a great advantage in reducing the exposure time to a few minutes, the difficulty experienced in keeping even approximately constant the light emitted from the carbon poles rendered its use unsatisfactory. For the purpose of detecting any inequalities produced on the plate by the source of light itself, a spectrum of the Nernst light was obtained on every negative. Cramer's spectrum plates were used throughout and as far as possible these were subjected to the same treatment in development. In the majority of cases blood was obtained from dogs but later experiments were made with blood from human patients without an anaesthetic.

RESULTS AND DISCUSSION

In a previous study (3) of the reaction of the blood flowing from different organs in the dog I had noticed that the blood from the adrenal vein invariably assumed a bright red arterial color in from one to twenty minutes after dilution with salt solution in different proportions, while blood flowing from other organs treated in exactly the same manner showed no change.

In casting about for an explanation of this change there occurred to me two possibilities which might be considered, viz., (1) that the color was due to some specific haemoglobin compound formed by the interaction with the haemoglobin of one or more substances flowing from the gland, or (2) to an increased formation of oxyhaemoglobin. As spectroscopic examination of adrenal vein blood possessing this arterial color revealed no spectra other than that of oxyhaemoglobin, the first possibility was at once dismissed. This finding, further, strongly indicated that the change in color was due to oxyhaemoglobin.

The next step, then, was to determine whether the blood from the adrenal vein would develop a greater amount of oxyhaemoglobin than venous blood from elsewhere in the circulation. A series of experiments showed without exception that the absorption bands of oxyhaemoglobin in adrenal vein blood were wider and possessed a greater intensity than those obtained from other venous blood of the same animal when both samples were withdrawn under the same conditions and diluted in the manner already described.

Photographs of spectra typical of the results obtained are reproduced in figures 1 and 2.

In the first of these blood was taken with a fine syringe from the adrenal vein, clamped at its opening into the vena cava immediately before the withdrawal of the blood, and from the aorta and vena cava at the same level. Immediately after withdrawal each sample of blood was diluted 1:10 with de-oxygenated Tyrode's solution in the manner already described. The oxyhaemoglobin absorption bands of the adrenal blood almost equal in width those of the aorta, while the spectrum of the vena cava blood possesses a much smaller amount of oxyhaemoglobin as indicated by the narrower bands. In figure 2 where blood withdrawn from the jugular vein and carotid artery and diluted 1:10 with 0.6 per cent NaCl is compared with blood from the adrenal vein similarly diluted, the oxyhaemoglobin content of the adrenal vein lies between that of the arterial and venous blood. However, in some cases the content of oxyhaemoglobin in the adrenal vein under approximately the same conditions except that another dilution medium was used, may occasionally be greater than that found in arterial blood at the same time (fig. 3). This is not infrequently the case where serum is used as a diluent.

Since Heubner and Rosenberg (4), Burns (5) and Butterfield (6) have shown conclusively that dilution, per se, does not play any rôle in increasing the absorption of oxygen by haemoglobin, and that Manchot's (7) conclusions to the contrary were largely due to faulty technique, this as a factor can be excluded.

Although the above dilutions gave the most favorable conditions for obtaining satisfactory spectra with not too long a time exposure, experiments were made with dilutions containing more than 10 per cent blood and it was found that up to a concentration of 25 per cent the results given above were confirmed. The increased opacity of concentrations higher than this, or of undiluted blood, necessitated the reduction of the thickness of the layer of blood to such a thin film in order to permit the penetration of rays of light that the concentration of the corpuscles in the film became abnormally reduced through capillarity, and unreliable results were obtained.

Dilution in vitro is, however, not necessary for the production of the arterial appearance as this frequently develops in the vein when the latter is clamped at its outlet into the vena cava. This fact was already noted by Arnaud (8) and later by Neumann (9).

The argument that the production of the arterial color in the vein is merely the expression of a decreased de-oxygenation of the blood passing through the gland is invalidated by the observation reported by

Neumann (9) that although the gland has an exceedingly rich blood supply its oxygen consumption per gram is equally high.

The above facts therefore indicate that the blood on its exit from the adrenal vein either possesses a greater amount of oxyhaemoglobin than venous blood from elsewhere in the circulation or the oxygen absorbing capacity of the haemoglobin which it contains has been greatly augmented.

Continuous secretion of adrenalin into the adrenal vein is a well authenticated fact. Further, the observation that the addition of adrenalin to the perfusion fluid favorably affects the oxygen intake of the heart in perfusion experiments (10) arouses a suspicion that this base may play a similar rôle in relation to the oxygen absorption of haemoglobin in the adrenal vein.

Will the addition of adrenalin to venous blood in any way modify its oxygen absorption?

Spectra reproduced in figures 4 and 5 demonstrate that adrenalin added in minute quantities does markedly accelerate the formation of oxyhaemoglobin in venous blood under favorable conditions.

To obtain these spectra a sample of venous blood was withdrawn from a dog and rapidly diluted in the proportions 1: 10 with Tyrode's solution in the one case and serum in the other. To one half of this diluted blood adrenalin in the concentrations indicated was added. That is, the blood diluted with serum contained adrenalin to saturation while to the specimen diluted with Tyrode's solution adrenalin in the proportion of 1: 100,000 was added. Both samples were immediately sealed in the spectroscopic absorption cells and spectra then obtained. Throughout the series wider absorption bands of oxyhaemoglobin in the specimen containing adrenalin always resulted. Further, other conditions remaining constant, the greater the amount of adrenalin added, the greater the percentage of oxyhaemoglobin formed. This is illustrated by figures 4 and 5. When the adrenalin was less in amount than 1: 100,000 the increase of oxyhaemoglobin was less than that shown in figure 5. This increase of oxyhaemoglobin as shown spectroscopically was correlated macroscopically with the assumption of an arterial color.

It is to be noted that the increased width of the absorption bands of oxyhaemoglobin produced by the addition of adrenalin may be influenced by factors other than the amount of adrenalin added. Thus, the amount of oxygen dissolved in the different solution media is a considerable factor in modifying the rate of oxygen absorption while

the chemical composition of the diluent is also of very great importance in this respect. With the same percentage of adrenalin and approximately equal amounts of available oxygen the widest bands were obtained when the blood was diluted with serum. Tyrode's solution gave the next widest bands, while the narrowest were obtained when sodium chloride was used. The variation in the ionic concentrations of these three dilution media and especially in their H^+ and OH^- content, probably accounts for considerable of the difference obtained. The identity of the spectra of adrenal vein blood with those obtained from arterial blood furnishes a proof that in the former case the absorption bands are oxyhaemoglobin.

Since characteristic physiologic effects could be obtained with the adrenalin base employed, in such quantities as 1: 1,000,000, the possibility of the presence to any extent of contaminating substances which might have given rise to the phenomenon above noted would seem to be precluded.

The similarity of the spectra of adrenal vein blood to those obtained from venous blood to which adrenalin was added is shown by a comparison of figures 1, 2 and 3 with figures 4 and 5. This similarity is unquestionably very strong evidence that it is adrenalin which is responsible for the increased amount of oxyhaemoglobin found in the adrenal vein blood.

These spectra also afford additional proof that the concentration of adrenalin in the adrenal vein, as calculated by previous investigators by other methods, lies in the neighborhood of 1: 1,000,000 (11).

Attention may be called to the fact shown in figure 5, spectrum 4, where the blood used was taken from the external saphenous vein of a dog without an anaesthetic, that the increase in the oxyhaemoglobin formed upon the addition of adrenalin to the blood from an animal without an anaesthetic is appreciably greater than in blood taken from the same animal under ether or chloroform. Thus it is evident that in the interpretation of experiments on the action of adrenalin in vivo anaesthesia is a factor which can not be disregarded.

Although the immediate action of adrenalin when added to a solution of venous blood is to increase the affinity of haemoglobin for oxygen and thus produce a larger percentage of oxyhaemoglobin, this is not a permanent effect. In absorption cells containing a mixture of adrenal vein blood and dilution medium in which the oxygen has been kept at a minimum, the bright color characteristic of oxyhaemoglobin was gradually replaced by a darker color of one of the other haemoglobin

compounds and frequently in four or five hours the oxyhaemoglobin was completely altered. The alteration taking place in the oxyhaemoglobin, as is seen in figure 5, spectrum 5, is not of the type of a reduction and nothing is known of the nature of the process occurring. Subsequently in this paper it will be referred to as a decomposition. In cases where the added dilution fluid contained any considerable quantity of oxygen in solution, the formation of the dark color was much delayed or failed to appear.

Although no special experiments were undertaken to study the factors involved in this reaction, the rapidity with which it developed depended in large degree upon oxygen being present in the solution in small amount only. Moreover, such factors as composition of diluent, temperature, amount of adrenalin added and amount of oxyhaemoglobin formed, all seemed to play a part in modifying the time required for the reaction.

An interesting observation to be recorded here is the retarding influence of ether on the development of the decomposition reaction. In blood from a dog without an anaesthetic in which the reaction of the blood is more alkaline (12) decomposition following the addition of adrenalin was marked at the end of an hour (provided the available oxygen of the dilution medium was limited) while in the same animal under anaesthesia to obtain a similarly intense reaction required at least four hours. This fact, together with that already mentioned, that the percentage of oxyhaemoglobin formed on addition of equal amounts of adrenalin is greater in blood from normal than from anaesthetized animals is a further indication of the importance of the reaction and composition of the blood as well as the amount of oxyhaemoglobin formed in governing this decomposition process. Macroscopically, this decomposed blood was of a mahogany color and microscopically showed complete disintegration of erythrocytes. Examination of absorption spectra of the decomposed substance disclosed the fact that the product ultimately resulting from the decomposition of the oxyhaemoglobin was urobilin. This substance gave a single absorption band in the green-blue region of the spectrum with a maximum intensity at λ 499 $\mu\mu$ (Lewin and Stenger) (13) and varying in width according to the density of the solution (figs. 5, 7 and 8).

Alkaline haematopophyrin has a spectrum similar to that of urobilin but in addition to the absorption band which it possesses in common with the latter compound, three faint narrow bands occur in the yellow and green. As no indication of these three narrow bands could be

obtained in any of the specimens, either by using a light of decreased intensity or by a shortened time exposure, the possibility of the substance being alkaline haematopophyrin is eliminated. The experiments throw no light on the mechanism of this process except that a direct transformation of urobilin takes place (fig. 5, spectrum 5).

Exposures thus far reported were made at room temperatures varying from 18°C. to 22°C. Bearing in mind Meyer's (14) conclusion, which was confirmed by Tribe (15) that the activity of adrenalin is much diminished at temperatures below that of the body, it does not seem unlikely that these chemical reactions obtained with adrenalin in vitro might be consummated in a considerably shorter period of time under normal conditions in the living organism. The temperature coefficient of haemoglobin is also an important factor in regulating the time of these reactions. The facts demonstrated by Mathison (16) and in greater detail by Oinuma (17) that in blood the rate of reduction is greatly accelerated and the rate of oxidation retarded by rise in temperature led to a series of experiments on human blood at a temperature of 38°C. The blood was taken from one of the median veins in the cubital space at the elbow without anaesthesia and diluted 1:10 with 0.85 per cent. NaCl at 38°C. under precautions similar to those already cited. The spectra, read directly from a smaller spectroscope in the constant temperature room confirmed in all points those obtained with dog's blood. It was not found possible, however, to reduce the time required for the reduction process giving rise to urobilin to less than one hour.

That compounds other than haemoglobin are not wanting in which adrenalin can bring about changes leading to an increased oxygen absorption is evidenced by the greater affinity of the heart for oxygen under the influence of this base. The existence of such compounds suggests that this phenomenon may be due to a general property of adrenalin rather than a specific reaction with various individual substances.

Now, results identical with those reported in this paper on adrenalin (increased oxygen saturation of haemoglobin) can also be obtained by addition of alkalies to the blood. Investigations of Barcroft (18) and his pupils have demonstrated that increased hydroxyl ion concentration is a very potent factor in augmenting the formation of oxyhaemoglobin. Kato (19) in a recent communication, shows that addition of alkali to blood to the extent of M/200 NaOH increases its oxidation 1.2 to 1.3 times the normal value, and further, that beyond a certain con-

centration, e.g., M/33 NaOH, this favorable action of alkalies on oxidation of the blood is lost.

In a previous paper (20) the observation was reported that the addition of adrenalin to serum or to venous blood was followed by a rise in the hydroxyl ion concentration.

The above facts led me to study in more detail the effect produced on the reaction of various fluids by adding adrenalin in definite proportions. From this study, which is not yet completed, I have taken two series of experiments on serum, the figures obtained are tabulated in the following columns.

Reaction of serum in pH before addition of adrenalin (base)*	*Increase in alkalinity, measured in millivolts, obtained on adding adrenalin (base)*
Sheep serum (fig. 9, curve 1)	
4.70	28
6.35	38
6.38	38
7.05	32
7.20	31
7.52	26
7.66	23
7.70	17
7.94	15
8.05	14
8.15	11
8.30	0
8.38	0
8.67	0
Dog serum (fig. 9, curve 2)	
4.70	26
5.05	31
5.85	32
6.17	30
6.51	25
6.82	22
6.85	16
7.54	11
7.88	8
8.67	0
9.01	0

* The reaction of each serum is given as pH, that is the common logarithm of the concentration of the hydrogen ions expressed as a positive instead of a negative number.

In the first of these sheep's serum was used. The hydrogen ion concentration of the normal serum was measured by the gas chain

method and to 25 cc. of this serum was then added to saturation adrenalin base. The fluid was then filtered and the reaction again determined. The normal serum was next made either more acid or more alkaline by adding varying amounts of normal HCl or normal NaOH and the acidity of each sample again measured. A number of sera, forming a series with an increasing hydrogen ion concentration was thus obtained. To 25 cc. of each modified serum of this series adrenalin

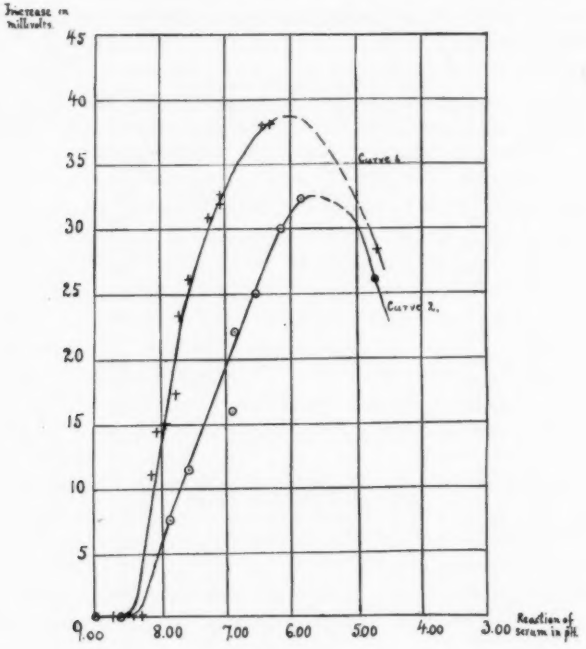

Fig. 9

base was added to saturation, and after filtering the reaction of each fluid was again determined.

In the second case the technique was the same except that dog's serum was used, and adrenalin was added in the proportion of 0.01 gram to every 25 cc. of serum.

When these results are plotted so that the abscissa represents the

reaction of the serum in pH and the ordinate denotes the increase in millivolts subsequent to the addition of adrenalin, curves are obtained similar to those reproduced in figure 9, curves 1 and 2. These curves are seen to resemble closely those obtained with amphoteric substances under similar conditions.

Briefly, these two curves show that the alkalinity of normal serum or of serum whose reaction is more acid than blood, is appreciably increased by the addition of adrenalin. In sera of which the values of the reaction lie between pH 6.50 and pH 8.00 this increased alkalinity is considerable and is proportional to the acidity. The values for the reaction of normal serum lie well within these two extreme figures which are only exceeded in certain pathological conditions (21). When adrenalin is added to defibrinated blood the changes are much less marked. I have not found it possible in defibrinated blood which was strongly oxygenated to elicit upon the addition of adrenalin any increase in alkalinity, though as previously stated, the addition of this base to fresh venous blood is followed almost immediately by an increase in alkalinity. This increase, however, is never such that the reaction of the blood which is obtained lies beyond the maximum hydroxyl ion concentration of arterial blood.

It may be noted that while the H^+ and OH^- ions exert the greatest influence upon the dissociation of adrenalin, other ions are not without effect. From the above two curves it is then evident that adrenalin does possess a property, viz., that of increasing the alkalinity of serum, which has also been shown by other investigators to influence favorably the oxygen absorption of haemoglobin. Therefore, the inference seems justifiable that a part at least, of the increased oxyhaemoglobin formation due to adrenalin can be explained on the basis of the increased alkalinity which this base produces.

Since it has been demonstrated that in man and dog addition of adrenalin to venous blood can give rise to increased hydroxyl ion concentration and simultaneously to increased oxyhaemoglobin production it may be asked what the importance of these facts are for the organism. Has the peculiar situation of the adrenal glands continuously pouring into the venous circulation, veritably on its last lap, a substance which has the property of increasing the alkalinity of venous blood, any physiological significance for the organs with which this fluid comes in contact? The stimulating effect of adrenalin on the heart when this substance is added to the perfusion fluid leaves no doubt as to its beneficial action on that organ. While it is very probable that adrenalin

possesses properties other than that of increasing the hydroxyl ion concentration of the blood, which may have a modifying influence on the action of the right heart, the increased alkalinity of fluids in its cavities, as shown by the experiments of Martin (22) and more especially those of Mines (23), would suffice to explain much of the increased tone which adrenalin is known to produce in that organ. If the formation of oxyhaemoglobin be looked upon as an oxidation process, then the enhanced affinity of haemoglobin for oxygen under the influence of adrenalin offers a striking parallel to the action of that substance on the heart for, as previously mentioned, Barcroft and Dixon (24) and others have shown that there is an increased consumption of oxygen by the perfused heart when adrenalin is added to the perfusion fluid. The similarity of the cited effects which adrenalin produces to that given by increased alkalinity unquestionably indicates the physiological importance of this property of adrenalin.

Further, this same property of adrenalin will not likely be less effective in the lungs. Since there is no reason to believe that the increased alkalinity which adrenalin produces to a more or less degree in the venous blood with which it comes in contact, will be lost before it reaches the capillaries of the lungs, it can still exert an influence in that organ. Accepting without any discussion as to the merits of the different methods of procedure employed, Haldane's (25) conclusions that under certain conditions the oxygen tension in the capillaries of the lungs is greater than that in the alveoli, it would seem probable that Haldane's interpretation of his results as being due to secretion of oxygen by the lung epithelium could be modified by the facts already noted in this paper. The presence of adrenalin in the venous blood of the capillaries of the lungs undoubtedly induces changes which meet the conditions suggested by Bohr (26) as requisite for an alternative to the explanation of oxygen secretion, that is, adrenalin could act as a substance altering the property of haemoglobin so as to give it a greater attraction for oxygen as it passes through the lung. To quote Haldane (27),

> increase of alkalinity would produce a considerable effect, but it is difficult to see how the alkalinity of the blood, or of the corpuscles, could rapidly change first in one direction and then in another as the blood passes through.

If what takes place in vitro is an index of what happens in vivo, then adrenalin could bring about these very conditions. Since Haldane's results were obtained with the CO method, the final criterion of the present hypothesis as a possible explanation of Haldane's facts, rests

upon whether the adrenalin would endow haemoglobin with an equally increased affinity for CO or oxygen. This, unfortunately, has not been determined. Again in his discussion of possible stimuli causing oxygen secretion in the lung, Haldane says, "want of oxygen is therefore, directly, or indirectly, a stimulus to oxygen." It would seem significant in this connection that Cannon (28) and his coworkers have shown that in asphyxia and in emotional disturbances, where there is urgent need of oxygen there is also a greatly increased secretion of adrenalin, and that Elliott's (29) results likewise indicate that in ether and chloroform anaesthesia, where the oxygen supply is depressed a depletion of the adrenalin occurs. If, as has been already assumed these 'in vitro' reactions can be applied to the living organism it is evident that the property which adrenalin possesses of increasing the hydroxyl ion concentration in blood will be very materially lessened, if not completely reduced, on the formation of oxyhaemoglobin in the capillaries of the lung, where adrenalin will encounter conditions most favorable for its oxidation. The advantage of such an adaption is obvious for if there existed in the arterial system any considerable amount of a substance which was capable of increasing the affinity of haemoglobin for oxygen, it would act detrimentally to the exchange of oxygen between the haemoglobin and the tissues. It is possible however, that in increased output of adrenalin by the adrenal glands a small part of this secretion escapes oxygenation in the lungs. That this is true in animals under urethane or ether anaesthesia has lately been demonstrated by Stewart (30).

The conclusion, therefore, that adrenalin does not exist normally in any but infinitesimal amounts beyond a very limited part of the circulatory system beginning with the adrenal vein and ending in the capillaries of the lung appears justifiable. It is conceivable that the need in other parts of the body for substances similar in action to adrenalin may be met by secretions from other glandular structures of a chromaffin nature (carotid gland, coccygeal body, etc.) which are adapted to their particular environment by slight differences in their physico-chemical properties.

SUMMARY

1. Blood from the adrenal vein of a dog, diluted to 10 per cent, 6.6 per cent and 5 per cent with normal saline, Tyrode's solution or dog's serum in which the dissolved oxygen is kept at a minimum, shows spectroscopically intensification of the oxyhaemoglobin bands.

2. Spectra obtained when adrenalin (base) is added to diluted venous blood are identical with those given by adrenal vein blood similarly diluted.

3. Venous blood to which adrenalin is added or adrenal vein blood forming an increase of oxyhaemoglobin when diluted show in from one to five hours, if the oxygen dissolved in the dilution medium be minimal, a transformation of the oxyhaemoglobin into urobilin.

4. Addition of adrenalin to diluted human venous blood causes an increase in the intensity of the oxyhaemoglobin absorption bands.

5. The possible physiological significance of these reactions for the heart and lungs in the living organism is discussed.

My sincere thanks are due to Prof. H. G. Gale, in whose laboratory the spectroscopic work was done, for instruction in technique and for his kind aid and advice throughout the course of the experiments. It is also a pleasure to acknowledge with many thanks my indebtedness to Prof. A. P. Mathews for suggestions and criticism during the progress of the work, and to express my grateful appreciation of Mr. Siegfried Maurer's kindness in assisting with many experiments.

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Fig. 1

Figures 1–8 are reproductions of photographs which have been reduced in size one-half.

Fig. 1. The numbers used in the explanation of this and the following figures refer to the spectra which each contains. These are numbered consecutively from above downward.

1. Spectrum of Nernst glower. Exposure 4 minutes.

2. Spectrum of adrenal vein blood, 10 per cent in 0.6 per cent NaCl. Exposure 1 hour.

3. Spectrum of jugular vein blood, 10 per cent in 0.6 per cent NaCl. Exposure 1 hour.

4. Spectrum of carotid artery blood, 10 per cent in 0.6 per cent NaCl. Exposure 1 hour.

5. Spectrum of iron arc. Exposure 45 seconds. The values of the wave lengths in Angström units are placed below various lines in this spectrum. The line having the value 5782 is due to a contamination of copper in the iron.

Fig. 2

1. Spectrum of Nernst glower. Exposure 5 minutes.

2. Spectrum of vena cava blood, 10 per cent in Tyrode's solution. Exposure 1¼ hours.

3. Spectrum of aorta blood, 10 per cent in Tyrode's solution. Exposure 1¼ hours.

4. Spectrum of adrenal vein blood, 10 per cent in Tyrode's solution. Exposure 1¼ hours.

5. Spectrum of iron arc. Exposure 45 seconds.

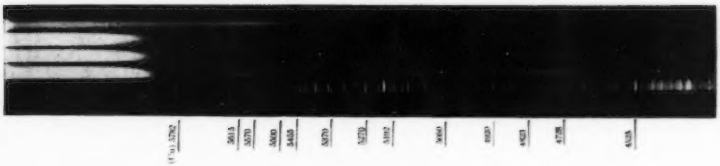

Fig. 3

1. Spectrum of Nernst glower. Exposure 5 minutes.
2. Spectrum of adrenal vein blood, 6.6 per cent in serum. Exposure 1¼ hours.
3. Spectrum of carotid artery blood, 6.6 per cent in serum. Exposure 1¼ hours.
4. Spectrum of jugular vein blood, 6.6 per cent in serum. Exposure 1¼ hours.
5. Spectrum of iron arc. Exposure 45 seconds.

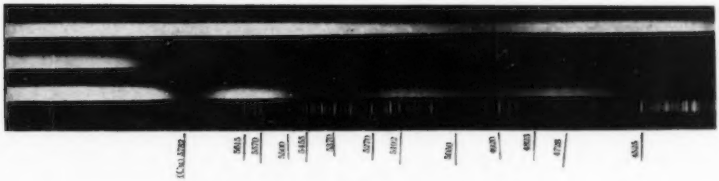

Fig. 4

1. Spectrum of Nernst glower. Exposure 5 minutes.
2. Spectrum of jugular vein blood, 10 per cent in serum plus adrenalin (saturation). Exposure 1¼ hours.
3. Spectrum of same sample of diluted blood but without adrenalin. Exposure 1¼ hours.
4. Spectrum of iron arc. Exposure 45 seconds.

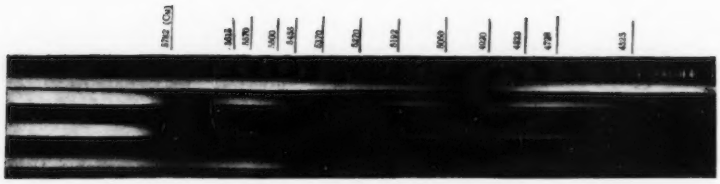

Fig. 5

1. Spectrum of Iron arc. Exposure 45 seconds.
2. Spectrum of Nernst glower. Exposure 5 minutes.
3. Spectrum of external saphenous vein blood, 10 per cent in Tyrode's solution. Exposure 1¼ hours. This blood was taken from a dog without an anaesthetic.
4. Spectrum of same sample of diluted blood plus adrenalin 1: 100,000 after standing 1¼ hours.
5. Spectrum of sample of previous blood (4) after standing 4 hours. Exposure 1¼ hours.

Fig. 6

1. Spectrum of vena cava blood, 10 per cent in 0.6 per cent NaCl. Exposure 1 minute.
2. Spectrum of same sample of diluted blood (1) plus adrenalin 1: 50,000. Exposure 1 minute.
3. Spectrum of vena cava blood, 20 per cent in 0.6 per cent NaCl. Exposure 3 minutes.
4. Spectrum of same sample of diluted blood (3) plus adrenalin 1: 50,000. Exposure 3 minutes.
5. Spectrum of vena cava blood, 10 per cent in 0.6 per cent NaCl. Exposure 2 minutes.
6. Spectrum of same sample of diluted blood (5) plus adrenalin 1: 50,000. Exposure 2 minutes.
7. Spectrum of continuous carbon arc. Exposure 5 seconds. At the point marked D in the original photograph there are two dark lines of the sodium spectrum at 5890 and 5896. Also, as the carbon contained traces of chromium three fine lines characteristic of this element occur at the point marked 5204.

Fig. 7

1. Spectrum of vena cava blood, 10 per cent in 0.6 per cent NaCl, to which adrenalin in the proportion of 1: 10,000 was added. Exposure 1¼ hours. The exposure was begun immediately after the blood was withdrawn.

2. Spectrum of the same sample of blood but exposure was begun 8 hours after the blood was withdrawn. Exposure 1¼ hours.

3. Spectrum of Nernst glower. Exposure 4 minutes. The iron arc exposure time was 2 seconds and the lines were so faint that they could not be reproduced.

Fig. 8

1. Spectrum of Nernst glower. Exposure 5 minutes.

2. Spectrum of vena cava blood, 10 per cent in 0.6 per cent NaCl plus adrenalin 1: 10,000. This was kept in the refrigerator for 24 hours and then exposed for 1 hour.

3. Spectrum of the same specimen of blood with an exposure of 30 minutes.

4. Spectrum of iron arc. Exposure 45 seconds.

THE EFFECT OF THE ACCUMULATION OF CARBON DIOXIDE ON THE TIDAL AIR AND ON THE H-ION CONCENTRATION OF THE ARTERIAL BLOOD IN THE DECEREBRATE CAT

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The experiments herein reported were performed: (1) in an attempt to produce in an acute experiment a definite change in the H-ion concentration of the arterial blood due solely to carbon dioxide; (2) to study the coincident response of the respiratory center and the changes in the total carbonate content of the arterial blood in an animal breathing gradually increasing percentages of carbon dioxide.

INTRODUCTION

Hasselbalch and Lundsgaard (1) and Lundsgaard alone (2) have demonstrated that the addition of CO_2 to blood *in vitro* will raise its C_H. Working with ox blood at 38.5° in gaseous equilibrium with gas mixtures containing CO_2 at different partial pressures, they found that the C_H of the blood increased as the partial pressure of carbon dioxide was raised.

Conversely, the removal of carbon dioxide from the blood causes a fall in C_H. Milroy (3) working with etherized dogs and cats found that a definite fall in the C_H of arterial blood resulted from forced pulmonary ventilation. Further, in two experiments in which the animals breathed gas mixtures rich in CO_2, he obtained a rise in the C_H of the blood.

With the exception of a few experiments reported by Hasselbalch and Lundsgaard (4), there are very few available data on the quantitative response of the respiratory center to CO_2 together with a determination of the C_H of the blood. This problem has an important clinical as well as physiological significance.

Many attempts have been made by clinicians to prove that certain types of dyspnea represent a condition of true carbon dioxide acidosis;

that is, are primarily due to retained carbonic acid rather than to other acids. These attempts have, however, been attended by very little success because indirect methods have been depended on for measuring the C_H of the blood. The difficulties involved in determining this value by the use of direct methods with sufficient accuracy to detect small deviations from the normal, has been the reason for the use of indirect methods. Such methods as the determination of the alveolar carbon dioxide, the total carbonate concentration of the blood and the ratio between these two, are in their present form not sufficiently accurate to have any particular advantage over the indicator method (5) in the detection of cases of carbon dioxide acidosis. Peters (6) in a recent paper has discussed the difficulties of determining the existence of carbon dioxide acidosis as the cause of cardiac dyspnea. The term "acidosis" as used in this paper signifies *an actual increase in the H-ion concentration of the blood,* whether or not it is detectable by methods at present available.

Although it is practically impossible to duplicate in animals the pathological conditions responsible for certain types of clinical dyspnea, nevertheless one can produce an accumulation of CO_2 and study the effect of the retained carbonic acid on the respiratory center, the C_H and the total carbonate content of the arterial blood. Given a respiratory center with normal sensitivity, it matters little what may cause an accumulation of CO_2; other things being equal, the effect on the center and on the C_H of the blood will be the same. Thus it is believed that the following experiments will help to throw some light on the question of carbon dioxide acidosis—a condition long thought to be responsible for certain types of dyspnea and hyperpnea, although there is no direct evidence that this is the case.

METHODS

Decerebrate cats were used in all the experiments herein recorded. The significance of a decerebrate preparation in studying the response of the respiratory center to CO_2 became evident in the early part of the work. In confirmation of the experience of other workers in this field, we were unable to find any anesthetic which did not depress the respiratory center if given in sufficient dosage to prevent struggling. In some experiments with urethanized cats, the respiration was apparently normal when breathing atmospheric air but when the animals were made to breathe increasing quantities of CO_2 in a closed system, the response of the respiratory center was quite unlike that given by a decerebrate cat under the same conditions. The latter responded to

gradually increasing percentages of CO_2 by a progressive increase in both rate and depth of respiration whereas the urethanized cat responded by an increase only in depth. The total increase produced in the tidal air for a given percentage of CO_2 was also different in the two cases. On account of the practical importance of these facts tracings of the response of the two preparations are shown in figures 1 and 2.

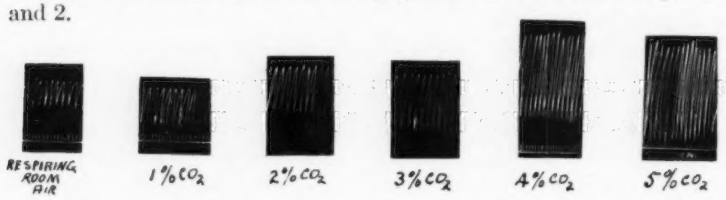

Fig. 1. The respiratory response to CO_2 in the decerebrate cat. Note the increase in rate as well as amplitude. When the CO_2 content of the inspired air was 5 per cent, the tidal air per minute was increased (414 per cent) over that with breathing room air. Upstroke in tracing represents expiration; downstroke, inspiration. Each millimeter = 4 cc. of air. Time in seconds.

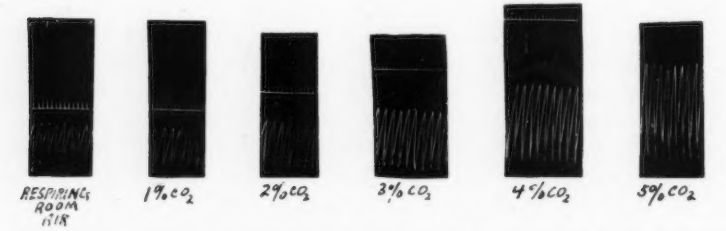

Fig. 2. The respiratory response to CO_2 in the urethanized cat (1.5 grams per kilo by stomach). Note the increase in amplitude but very little in rate. When the CO_2 content of the inspired air was 5 per cent, the tidal air per minute was increased 214 per cent over that when breathing room air. Upstroke in tracing represents expiration; downstroke, inspiration. Each millimeter = 4 cc. of air. Time in seconds.

It is noted that when the decerebrate cat was breathing an atmosphere of 5 per cent CO_2, there was an increase amounting to 414 per cent in the tidal air per minute. That is to say, the animal was respiring 414 per cent more air than when breathing atmospheric air. Under the same conditions the urethanized cat had increased pulmonary ventilation by only 214 per cent.

That anesthetics depress the respiratory center is no new observation. Ample evidence of this fact is had from the work of Cushny and Lieb (7) and Cushny (8) alone. They found that rabbits under light urethane anesthesia had a high CO_2 content of the arterial blood. The average percentage of CO_2 in the carotid blood of three decerebrate rabbits was found to be 31.2 per cent. In nine animals receiving 1 gram urethane per kilo subcutaneously, the CO_2 content of the arterial blood averaged 50.5 per cent. Such high concentrations in decerebrate rabbits produced marked hyperpnea. It is evident from these observations that even small doses of urethane so depress the respiratory center that it will tolerate a much higher percentage of CO_2 than will a normal center. Michaelis and Davidoff (9) have also observed that anesthetics render the respiratory center less sensitive to elevations in the C_H of the blood. Newburgh, Means and Porter (10) have recently reported results showing the depressing effect of urethane and ether upon the respiratory reaction to CO_2. They point out too the depression caused by anesthetics in general. In an unanesthetised dog trained to breathe increasing quantities of CO_2 in a closed system, they obtained practically the same respiratory response as in decerebrate dogs thus showing that the decerebration in itself does not appreciably depress the center if sufficient time is allowed for the elimination of the anesthetic. From such observations it is evident that the sensitivity of the respiratory center is a very important factor in maintaining at its normal level the molecular ratio $\frac{H_2CO_3}{NaHCO_3}$ and consequently the C_H of the blood.

The method of decerebration employed was the one devised in Sherrington's laboratory. A tracheal cannula is inserted with the animal under ether anesthesia, both carotids are clamped and the posterior vertebral arteries are compressed from behind. The animal is now placed on the decerebrator and the anterior portion of the head removed. (Figure 3 shows the section through the brain and the part of the head removed by the operation.) This gives a sharp and clean section passing in front of the tentorium cerebelli through the posterior part of the midbrain and emerging about a millimeter anterior to the front edge of the pons. After decerebration the preparation is removed to the experiment table and compression of the vertebrals continued for a few minutes—or until their release causes no bleeding. Later the carotids are opened. Immediately following decerebration the animal may stop breathing but after artificial respiration for a few seconds

spontaneous breathing develops and continues in a perfectly normal manner. The blood pressure falls immediately following the decerebration but rapidly returns to normal. For the exact details of the method the paper by Miller and Sherrington (11) is cited.

In our experience the above procedure gave far more satisfactory results than the older method of trephining the skull and separating the pons with a blunt instrument. However carefully this operation was done, different animals had wide variations in their respiratory rate and likewise marked variations in their response to CO_2. On hemisection of the head there was invariably found a blood clot around the medulla.

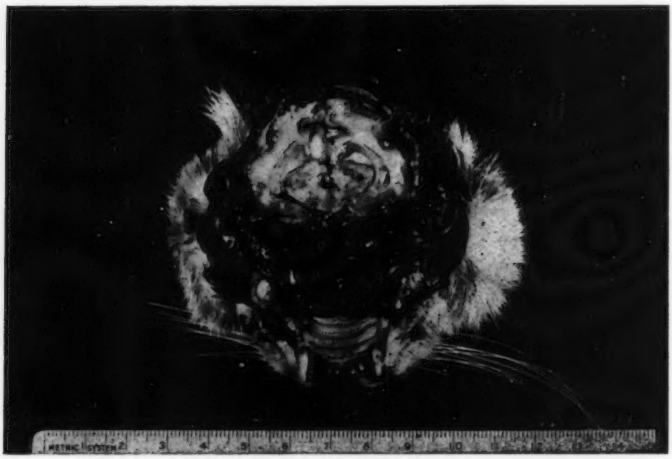

Fig. 3. Showing the section through the brain and the portion of the head removed by the operation for decerebration in the cat.

After decerebration an interval of one hour was allowed for recovery from ether. (During this time and throughout the experiment care was taken to prevent loss of body heat so that the rectal temperature never varied more than 1°C.) The tracheal cannula was now connected to a two-way valve[1] so arranged that the animal inspired from a bottle and expired into a Gad aeroplethysmograph equipped with a writing

[1] This valve is so constructed that it affords a minimum impediment to respiration. It was designed by R. G. Pearce and will be described by him in a future communication.

style to record on a kymograph. A short piece of rubber tubing connected the aeroplethysmograph with the bottle, thereby forming a closed system.[2]

Samples of the air in the bottle were drawn off by glass ground syringes equipped with three-way stopcocks similar to those of the Haldane sampling tubes. The CO_2 analyses of the bottled air were made with the Haldane apparatus. Before each experiment the system was filled with atmospheric air and during it sufficient oxygen was added to maintain the percentage of this gas to at least 14. Since any slight impediment to respiration will increase the minute volume, the animal was allowed to breathe through the valves for five minutes before every experiment.

The hydrogen-ion determinations on the blood were made with the dialysis indicator method (5). The blood was allowed to flow from a cannula in the femoral artery directly into the bottom of a collodion sac. The sac had previously been put into a "non sol" tube[3] containing 3 cc. of isotonic neutral (tested) NaCl solution. When about 1.5 cc. of blood was collected, the sac was lowered and the tube stoppered. (It is believed that the above technique reduced the loss of CO_2 to a minimum.) Ten minutes were allowed for dialysis. Phenolsulfonephthalein was added and the resulting color compared with standards prepared from phosphate mixtures calibrated by the gas chain method. No effort was made to read closer than 0.05. If for example a color fell between P_H 7.4 and P_H 7.5, it was read as 7.45. Usually three C_H determinations of the blood were made in each experiment. The first sample of blood was taken while the animal was breathing atmospheric air through valves; when the CO_2 in the bottle had risen to a high percentage (5 to 7 per cent), the second sample of blood was taken; after the animal was disconnected from the apparatus and had been breathing atmospheric air for fifteen minutes, the third sample was taken.

In a few experiments the total CO_2 content of the arterial blood was determined by the Barcroft-Haldane method. Blood was allowed to flow directly from a cannula in the femoral artery into a 1 cc. graduated pipette containing a few oxalate crystals (free from carbonate). One-half cubic centimeter was immediately delivered under ammonia water into one bottle of the original Barcroft-Haldane apparatus.

[2] This method was designed by Haldane and first employed with animals by Newburgh, Means and Porter (9).

[3] The tubes were of uniform bore (1 cm.).

The bottle was gently shaken to lake the blood, 0.25 cc. of a saturated solution of potassium ferricyanide then added, and the bottle again shaken to expel all the free O_2. Connection was then made to one of the manometers, the other manometer being connected with a bottle containing the reagents to serve as a dummy. After the temperature had become constant, 0.25 cc. saturated citric acid was spilled from the spoon into the bottle and the CO_2 thus liberated determined in the usual manner. The samples of blood were taken at the same time as those for the determination of the C_H.

RESULTS

Figure 4 is a typical record obtained with the technique described above. It shows the quantitative response of the respiratory center to gradually increasing quantities of CO_2, the data secured from the

TABLE 1

The respiratory reaction to carbon dioxide in the decerebrate cat

CAT NUMBER	WEIGHT	EXPERIMENT NUMBER	INITIAL RESPIRATION PER MINUTE	INITIAL VENTILATION PER MINUTE	PERCENTILE INCREASE IN MINUTE VOLUME AS THE INSPIRED CO_2 INCREASED FROM 1 TO 6 PER CENT*					
					1 %	2 %	3 %	4 %	5 %	6 %
	kgm.			*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
20†	1.8	1	26	836	25	65	140	255	450	600
20		2	25	624	25	75	160	386		
20		3	26	728	25	80	175	310	500	610
20		4	28	754	20	75	165	315	390	
20		5	26	728	30	80	170	315	465	600
21	1.3	6	20	576	10	30	75	135	210	300
21		7	20	504	25	60	100	160	250	350
21		8	20	560	15	45	80	150	240	350
21		9	25	500	30	75	135	215	325	440
22	2.1	10	35	910	32	80	150	240	330	400
23	2.3	11	27	864	30	85	145	270	440	575
23		12	30	1,080	20	65	130	260	360	400
24	2.2	13	28	840	15	35	75	140	230	400
25	3.5	14	24	1,056	35	100	150	325	475	
26	2.3	15	39	1,248	20	50	95	150	220	300
27	3.1	16	24	960	10	35	75	150	250	360
Average.....			26	800	23	64	126	236	342	437

* The CO_2 percentages in the samples of the inspired air were not always whole numbers. Consequently some of these data were obtained by interpolation.

† This cat was decerebrated at 9 a.m. At 5.30 p.m. experiment 5 was done. Note the response to CO_2 was practically the same as in the earlier experiments.

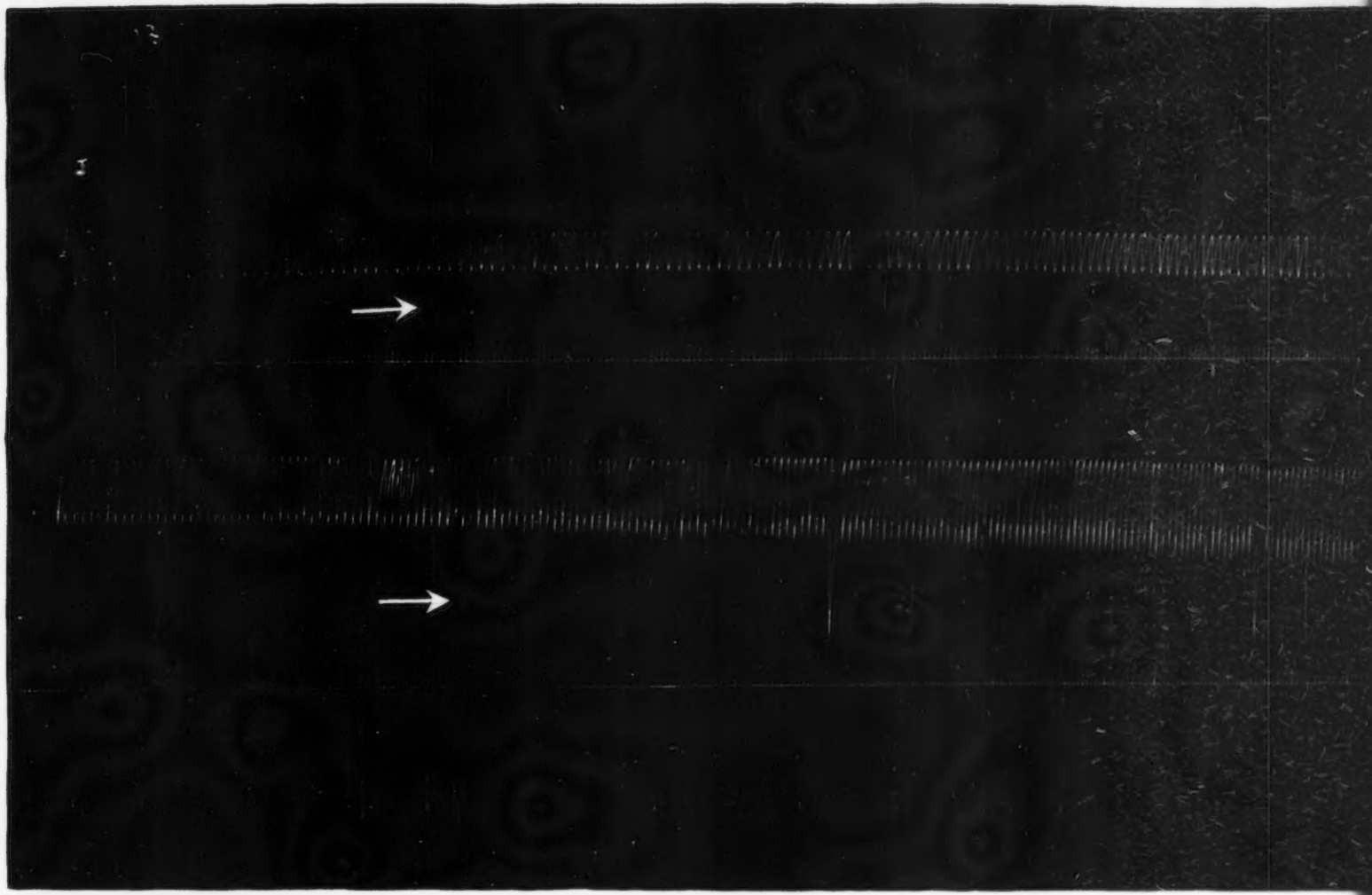

Fig. 4. The quantitative response of the respiratory center to carbon dioxide in the decerebrate cat. The tracing represents the tidal air as CO_2 accumulated fr

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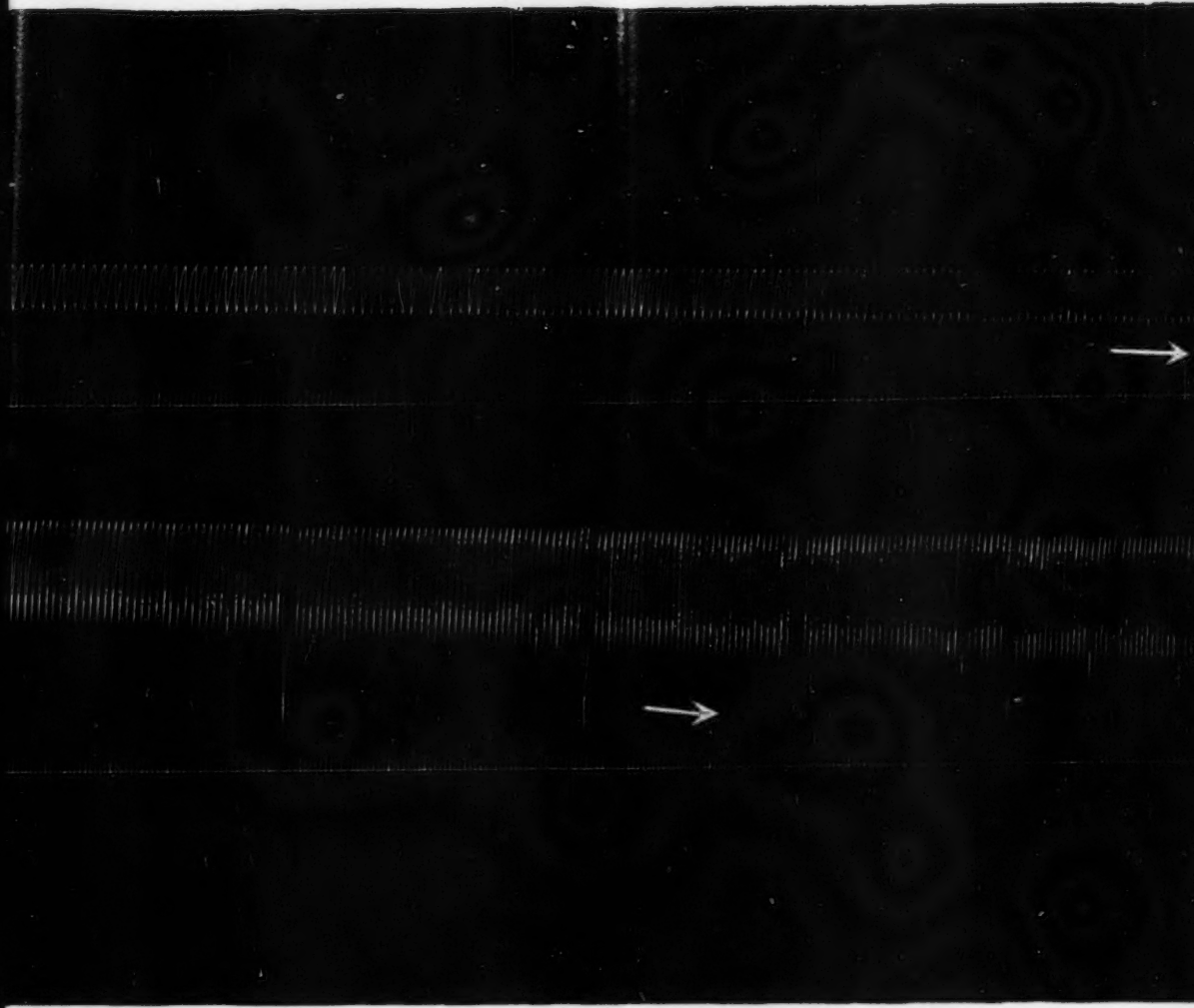
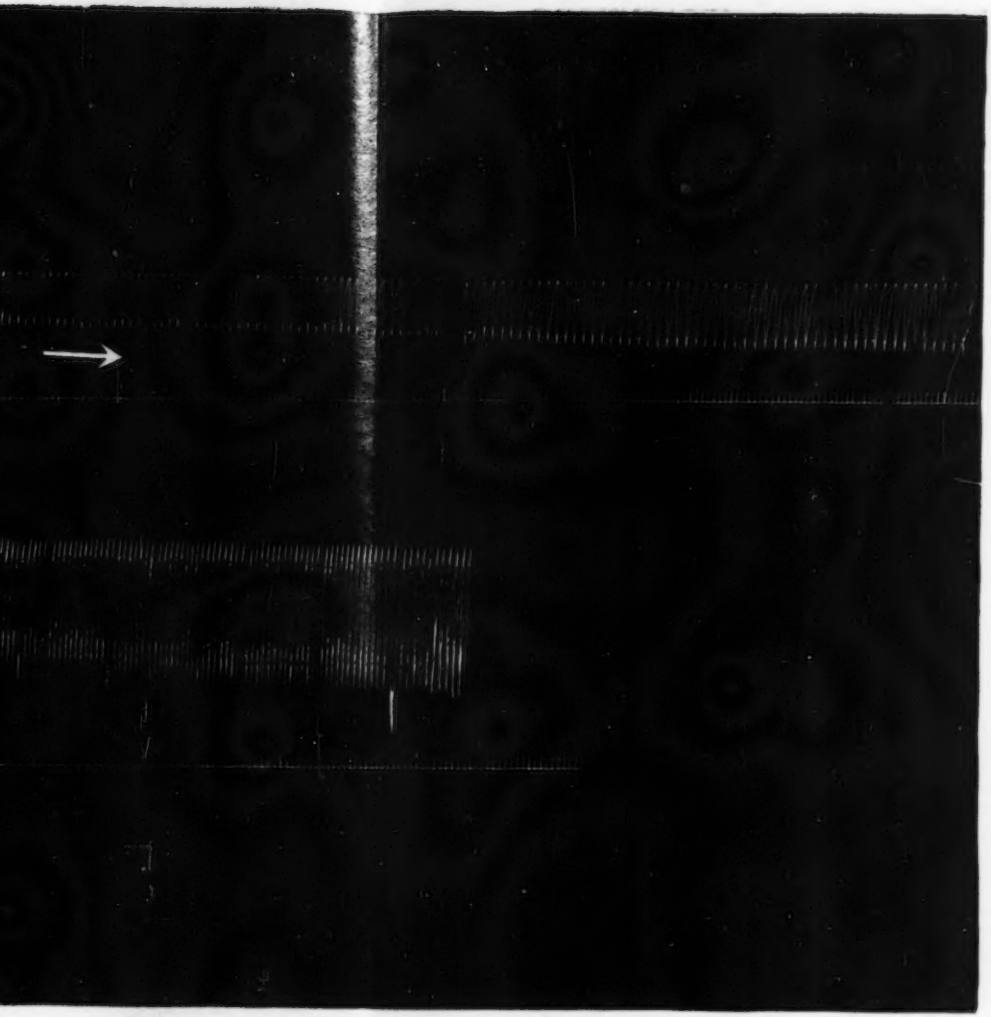
RESPIRING ATMOSPHERIC AIR		
Respirations per minute	Tidal air per respiration	Tidal air minute
	cc.	cc.
36	24	864



presents the tidal air as CO_2 accumulated from 0.04, the percentage in the room air to 7.2 per cent. Upstroke represents expirati

Data from figure 4

RESPIRING ATMOSPHERIC AIR			RESPIRING AIR CONTAINING $\begin{cases} CO_2 = 7.3\% \\ O_2 = 15.1\% \end{cases}$			
Respirations per minute	Tidal air per respiration	Tidal air per minute	Respirations per minute	Tidal air per respiration	Tidal air per minute	Percentile increase in tidal air per minute
	cc.	*cc.*		*cc.*	*cc.*	*per cent*
36	24	864	60	90	5400	525



sents expiration, downstroke inspiration. Each millimeter = 4 cc. of air. Time in seconds.

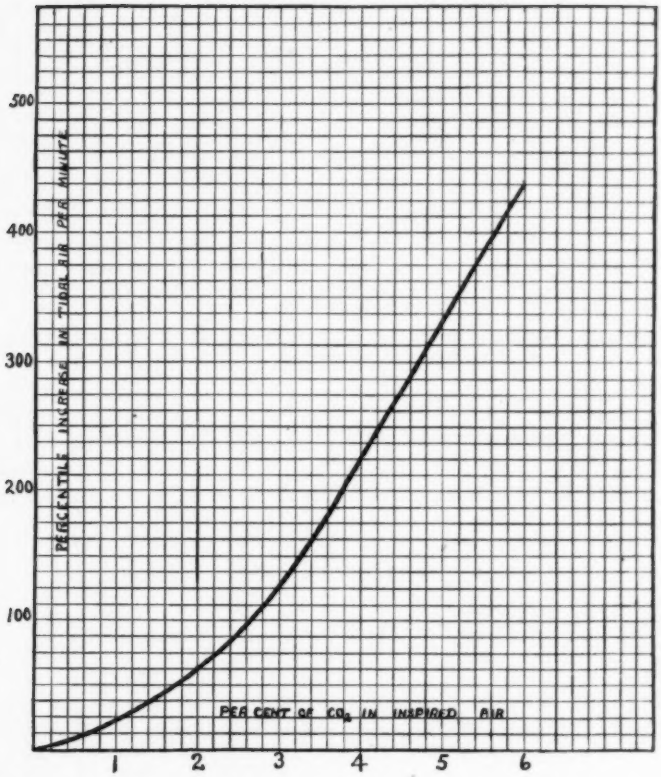


Fig. 5. Composite curve obtained from the data on sixteen experiments, showing the respiratory response to CO_2 in the decerebrate cat. Abscissae = percentage of CO_2 in the inspired air. Ordinates = the percentile increase in the tidal air per minute.

tracing being added to the legend. The results computed from similar tracings are expressed in tabular form in table 1. If for any reason the respiratory function seemed to be affected by the decerebration, the animal was discarded. For each experiment a curve was also made in which the percentile increase in tidal air per minute was plotted as ordinates, and as abscissae the actual percentages of CO_2 found to be contained in the sample taken from the bottle from which the

animal inspired. Usually six samples were withdrawn during each experiment. From such a curve the figures for the percentile increase in tidal air per minute were interpolated. Figure 5 is a composite curve obtained from the data of table 1.

Our results differ somewhat from those reported recently by Newburgh, Means and Porter (10) on cats. These workers record variations in the initial respiratory rates in different animals varying from 6 to 82 per minute, which can scarcely be considered normal. Also their data on the respiratory response to CO_2 in different cats show somewhat wider discrepancies than we have obtained. In our experience the

TABLE 2

The effect of rebreathing carbon dioxide on respiration and on the hydrogen-ion concentration of the arterial blood in the decerebrate cat

CAT NUMBER	PRELIMINARY PERIOD*							REBREATHING PERIOD†						FIFTEEN MINUTES AFTER REBREATHING PERIOD‡
	Weight	Experiment number	Time	Temperature per rectum	Respiration per minute	Tidal air per minute	P_H arterial blood	Respiration per minute	Tidal air per minute	Percentile increase in tidal air per minute	CO_2 in inspired air	O_2 in inspired air	P_H arterial blood	P_H arterial blood
	kgm.					*cc.*			*cc.*	*per cent*	*per cent*	*per cent*		
13	2.3	17	2.35 p.m.	38.7°	37	884	7.45	56	4,256	381	6.21	13.90	7.25	7.4
13		18	3.10 p.m.	38.8°	35	840	7.45	59	5,428	546	6.90	14.02	7.20	7.45
27	3.1	19	11.15 a.m.	39°	24	960	7.4	48	6,528	580	8.23	14.10	7.15	7.4
26	2.3	20	10.30 a.m.	39°	39	1,248	7.4	63	5,040	304	6.21	14.00	7.25	7.4
24	2.2	21	1.45 p.m.	38.5°	31	930	7.4	54	5,124	451	6.12	18.12	7.20	7.4
24		22	2.30 p.m.	38.7°	28	840	7.4	50	4,200	400	6.00	18.00	7.25	7.45
6	1.3	23	10.50 a.m.	38.8°	18	576	7.4	35	2,520	337	7.00	30.00	7.20	7.4
6		24	11.30 a.m.	38.8°	18	504	7.4	42	3,360	566	7.52	13.51	7.20	7.4
6		25	12.20 p.m.	39°	20	560	7.45	45	3,420	500	6.85	15.12	7.25	7.4
11	2.3	26	11.45 a.m.	39°	27	864	7.4	40	5,600	548	6.75	13.15	7.25	7.4
Average					27.7	820	7.41							

* Animal respiring room air through valves.

† Animal respiring in a closed system, thus accumulating carbon dioxide.

‡ Animal disconnected from closed system and respiring room air through valves.

variations in both the initial respiratory rates and the respiratory responses to CO_2 are to be attributed to the method of decerebration which they employed.

In table 2 are given the results showing the respiratory response and the changes in the P_H of the arterial blood produced by breathing relatively high percentages of CO_2. As will be noted, the oxygen percentages varied considerably. In the earlier experiments the oxygen was allowed to fall to 13 or 14 per cent. It might be contended that

TABLE 3

The effect of rebreathing carbon dioxide on the minute volume and on the H-ion concentration and total carbonate content of the arterial blood in the decerebrate cat

EXPERIMENT NUMBER	PRELIMINARY PERIOD*								REBREATHING PERIOD						FIFTEEN MINUTES AFTER REBREATHING PERIOD†	
	Cat number	Weight in kgm.	Time	Temperature per rectum	Respiration per minute	Tidal air per minute	P_H arterial blood	Total CO_2 content of arterial blood per 100 c.c.	Respiration per minute	Tidal air per minute	Percentile increase in tidal air per minute	CO_2 in inspired air	P_H arterial blood	Total CO_2 content of arterial blood per 100 cc.	P_H arterial blood	Total CO_2 content of arterial blood per 100 cc.
		kgm.				*cc.*		*cc.*		*cc.*	*per cent*	*per cent*		*cc.*		*cc.*
27	36	2.2	10.30 a.m.	38.5	48	864	7.45	30.3	64	3,968	540	6.45	7.25	53.4	7.4	34.2
28	33	2.2	1.50 p.m.	38.8	22	616	7.40	40.2	40	2,880	367	5.02	7.20	58.0	7.35	42.0
29	35	2.0	10.30 p.m.	38.6	28	784	7.40	38.1	40	3,840	389	5.35	7.25	51.1	7.4	40.0
30	32	2.3	11.00 p.m.	38.7	32	768	7.45	30.2	54	4,536	489	6.40	7.20	51.1	7.4	35.0
31	31	2.0	11.15 a.m.	38.8	32	896	7.40	32.0	60	4,800	436	5.95	7.20	50.0	7.4	35.2
	Average				32.4	785	7.42	34.1								

* Animal respiring room air.

† Animal disconnected from system and breathing room air for fifteen minutes.

with such low percentages of oxygen, acid products of incomplete combustion were added to the blood and thus helped to raise the C_H. Accordingly a few experiments were done in which, by adding oxygen to the bottle, the oxygen percentage of the rebreathed air was kept as high, or even higher than that of the atmospheric air. As the results show, the increase in the C_H was of the same magnitude whether the oxygen percentage in the inspired air was 13.15 or 30 per cent.

Further evidence of the accumulation of CO_2 in the blood is afforded

by the data given in table 3, showing the effect of breathing relatively high percentages of CO_2 on the minute volume of the air breathed and on the C_H and total carbonate content of the blood. It is noted that the carbonate content averaged 34.1 per cent in five decerebrate cats. When these same animals inspired air containing from 5.02 to 6.45 per cent CO_2, the total carbonate content was increased to from 51 to 58 per cent.

DISCUSSION

With a gradually increasing percentage of CO_2 in the inspired air, a point is reached where in spite of the accelerated respiration sufficient H_2CO_3 accumulates in the blood to produce a detectable elevation in the C_H. Accompanying this increase in H_2CO_3, there is a very considerable elevation in the bicarbonate content above normal. The magnitude of the elevation in both H_2CO_3 and $NaHCO_3$ may be had from the following calculations based on the data obtained in experiment 27 on cat 36.

TABLE 4

(Experiment 27)

The effect of rebreathing carbon dioxide on the free and combined CO_2 in the arterial blood of the decerebrate cat

	P_H	C_H	$(H_2CO_3)+(NaHCO_3)$	$\frac{(H_2CO_3)}{(H_2CO_3)+(NaHCO_3)}$ *	(H_2CO_3)	$(NaHCO_3)$
			cc. per 100 cc.		*cc. per 100 cc.*	*cc. per 100 cc.*
Preliminary period	7.45	3.5×10^{-8}	30.3	0.046	1.4	28.9
Rebreathing period	7.25	5.6×10^{-8}	53.4	0.071	3.8	49.6

* $\frac{\lambda C_H}{K + \lambda C_H} = \frac{0.605\, C_H}{4.4 \times 10^{-7} + 0.605\, C_H}$. In this equation λ = degree of dissociation of $NaHCO_3$ under the conditions existing in blood; k = dissociation constant of H_2CO_3. The values used are those given by Michaelis and Rona (Biochem. Zeitschr., 1912, xliv, 232).

During the preliminary period while the animal was respiring atmospheric air, the molecular ratio $\frac{H_2CO_3}{NaHCO_3}$ of the arterial blood was $\frac{1.4}{28.9}$. At the end of the rebreathing period, when the CO_2 in the in-

spired air was 6.45 per cent, the ratio was $\frac{3.8}{49.6}$. Although there was an absolute increase in the blood bicarbonate from 28.9 to 49.6 in terms of volumes per cent carbon dioxide, the ratio $\frac{H_2CO_3}{NaHCO_3}$ was increased from 1/20.6 to 1/13.[4]

It was shown many years ago by Zuntz (12) that the introduction of CO_2 into blood raises not only the H_2CO_3 but also the $NaHCO_3$ content, by such reactions as $Na_2HPO_4 + H_2CO_3 = NaH_2PO_4 + NaHCO_3$. Any condition therefore which causes an abnormal retention of CO_2 in the organism must lead to disturbances in the chemical equilibria in the blood in the direction indicated by our results. Such conditions fall under four general heads. They may act singly or any combination of the four may be responsible.

1. Increase in general body metabolism; e.g., fevers, physiological exercise.

2. Lowered sensitivity of the respiratory center; e.g., narcosis, comatose states, etc.

3. A high percentage of CO_2 in the inspired air.

4. A failure on the part of the organism to eliminate an adequate quantity of CO_2—resulting from:

a. A poor mass movement of blood through the lungs, as in heart disease.

b. Some interference with the passage of CO_2 from the venous blood to the alveolar air.

c. An impediment to the mechanics of respiration thereby causing an interference with the free ingress and egress of air.

The above data indicate that we are dealing with a condition of true carbon dioxide acidosis. When our animals inspired an atmosphere containing 5 to 6 per cent CO_2, the increased activity of the respiratory center was inadequate to maintain the molecular ratio $\frac{H_2CO_3}{NaHCO_3}$ at a normal level. The accumulated H_2CO_3 was to a large extent converted to $NaHCO_3$, yet there was still a sufficient elevation in the ratio $\frac{H_2CO_3}{NaHCO_3}$ to produce a demonstrable elevation in the C_H of the blood. If we accept the present hypothesis that the C_H of the blood is alone concerned in the chemical regulation of breathing, it follows that the first appreciable increase in the tidal air in the above experiments must have

[4] The writer is indebted to Dr. C. H. Fiske for these calculations.

been due to an elevation in the C_H of the blood of such a magnitude that, although it was sufficient to stimulate the respiratory center, it could not be detected in the blood itself by any means at our disposal. The results of Haldane and his coworkers on man are usually interpreted in the same way. They found that an increase in C_H of only 0.005×10^{-7} was sufficient to cause an increase of 50 per cent in the alveolar ventilation.

Much has been written in recent years on the subject of acidosis but there is still some disagreement over the definition of the term. No doubt much of the confusion is due to a failure to distinguish between acidosis and detectable acidosis. As defined by some authors, acidosis is a condition in which the bicarbonate in the blood is reduced below its normal level. Such a definition applies to the condition in which the acids responsible for the elevation of C_H are some other than H_2CO_3, as in diabetes, nephritis, etc. Such a definition does not consider carbon dioxide acidosis in which, as shown by our data, there is an increase in the bicarbonate content of the arterial blood above normal.

That it is possible to have a depletion of blood bicarbonate without an actual increase in the C_H, seems a debatable question. Koppel and Spiro (13) have shown that the buffer value of a solution is directly proportional to the concentration of buffer substances in the solution. Accordingly, in so far as the blood bicarbonate is depleted below normal, the addition of the same amount of acid must produce a proportionally greater elevation in the C_H than in normal blood. In other words, the minute fluctuations in C_H that must occur under strictly normal conditions would simply be exaggerated, and in spite of the accelerated respiration would remain so until the bicarbonate was restored to its normal value. Assuming that even the normal quantity of acid products of metabolism is being added to the blood depleted in bicarbonate, it is evident that there must occur a greater elevation in C_H than would take place under the same conditions with normal blood. The respiratory center is stimulated and lowers the H_2CO_3 thereby reducing to a more normal proportion the ratio $\frac{H_2CO_3}{NaHCO_3}$, consequently the C_H of the blood is maintained within normal limits so far as our methods, at present available, are able to detect. If by any accelerated respiration the molecular ratio $\frac{H_2CO_3}{NaHCO_3}$ is maintained at approximately its normal level in the arterial blood, then there must be some factor responsible for the increased respiratory activity. In the light

of our present knowledge it can only be a slight elevation in the C_H of the blood. Because this increase in C_H is not detectable is no evidence that it is not active.

Barcroft (14) has shown that the dissociation curve of oxyhemoglobin is extremely sensitive to slight changes in reaction. In persons living at high altitudes he was unable to detect any change in the curve provided the pressure of CO_2 in the blood is reduced to that of the alveolar air at the same altitude. This seems to indicate that the C_H within the body remains unaltered. Contrary evidence, however, is afforded by the observations made on Pike's Peak by Haldane, Douglas, Henderson and Schneider (15). They found that with an unaltered respiratory exchange and a sensibly unaltered dissociation curve of oxyhemoglobin, the alveolar ventilation was approximately 50 per cent greater than at sea level. This increased activity of the respiratory center cannot be accounted for by the direct action of oxygen want, as shown by Boycott and Haldane (16). From these observations we can conclude that the accelerated alveolar ventilation at high altitudes is due to a slight elevation in the C_H of the blood. When one considers that the respiratory center is by far the most sensitive index of C_H known, it is not surprising that it would be stimulated to increase pulmonary ventilation by an acid increment in the blood far too small to be demonstrable by our most refined methods of measurement.

References to the data of table 1 shows that 2 per cent CO_2 in the inspired air caused an average increase of 64 per cent in the tidal air per minute. Examination of the arterial blood at this stage of the experiment would reveal no demonstrable elevation in the C_H, but an appreciable increase in the total carbonate content. In this case, in so far as the accelerated respiration was adequate to maintain the C_H normal within detectable limits, we can call the condition one of compensated acidosis, but there is no evidence to justify the conclusion that there was not an actual elevation in C_H responsible for the increased respiratory activity. Much the same argument holds for those conditions in which the bicarbonate content of the blood is reduced.

The definition of acidosis as given in the early part of this paper seems to consider all the facts. When due to CO_2, the bicarbonate content of the blood is elevated; when caused by acids other than H_2CO_3 the bicarbonate is diminished, as shown by the work of many investigators, notably Van Slyke and his coworkers (17). It is probable that in certain conditions, e. g., heart disease, both CO_2 and fixed

acids may be responsible. In either general type, if the regulatory mechanisms are normally active, gross changes in the blood bicarbonate must occur before there is any detectable elevation in the C_H. In mild clinical acidosis and in persons living at high altitudes, a slight increase in C_H is to be considered as a result of a physiological response. If, as assumed by some authors, a demonstrable increase in C_H of the blood is an indication of failure of the regulatory function (uncompensated acidosis), then such an index is purely arbitrary—fixed by the sensitivity of our present methods to detect slight deviations in the C_H above the normal.

SUMMARY

1. The sensitivity of the respiratory center is a very important factor when one is studying the respiratory response to changes in the acid-base equilibrium of the blood.

2. To avoid the depressing effects of anesthetics on the sensitivity of the center, a decerebrate preparation may be used.

3. When decerebrated cats are made to breathe through valves in a closed system, the pulmonary ventilation increases in volume as the CO_2 accumulates in the system.

4. When the inspired CO_2 has reached about 4 per cent, an increase in the C_H of the arterial blood becomes detectable by colorimetric methods. With the O_2 in the inspired air varying between 13.15 and 30 per cent, like percentages of CO_2 produce the same elevation in the C_H of the blood.

5. Following a rebreathing experiment in which there has occurred a demonstrable elevation in the C_H, breathing room air for fifteen minutes restores it to normal.

6. Although a small increase of CO_2 (2 per cent) in the inspired air causes a conspicuous acceleration of pulmonary ventilation, no change in the C_H can be detected in the arterial blood. This indicates that the respiratory center responds to slight elevations in C_H that are too minute to be demonstrable by the method employed.

7. Accompanying the rise in C_H is a marked increase in the total carbonate content of the arterial blood. By breathing room air for fifteen minutes the total blood carbonate is restored almost to its original value.

8. When breathing 5 to 6 per cent CO_2 the accelerated respiration fails to maintain the ratio $\frac{H_2CO_3}{NaHCO_3}$ at its normal level. Although

the accumulated H_2CO_3 is to a large extent converted to $NaHCO_3$, the ratio $\frac{H_2CO_3}{NaHCO_3}$ is elevated sufficiently to cause a demonstrable rise in the C_H of the blood.

9. In acidosis due to acids other than CO_2, the bicarbonate content of the blood is reduced below normal. Evidence is presented to indicate that when the blood bicarbonate is diminished there is an actual increase in the C_H of the blood.

10. There is an increase in the C_H as well as an elevation in the bicarbonate of the arterial blood in acidosis due to the retention of carbon dioxide.

11. The definition that acidosis is a condition in which there is an actual increase in the H-ion concentration of the blood is the only one that is sufficiently comprehensive.

The writer is indebted to Mr. D. H. Hoover for assisting in the air analyses and to Mr. Edward Warnick for valuable technical assistance.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, NO. 295.

THE SKIN OF THE CATFISH (AMIURUS NEBULOSUS) AS A RECEPTIVE ORGAN FOR LIGHT

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Among the vertebrates numerous amphibians and some reptiles have already been shown to have a skin whose nerve endings are sensitive to light. In fishes, however, such cases are rarer. Eigenmann (1) in Chologaster and in Amblyopsis, Parker (2) in Ammocoetes, and Payne (3) also in Amblyopsis, have observed light reactions due to the nerve endings in the skin. Parker (4) has listed a number of marine fishes from which no integumentary light responses could be obtained.

Amiurus nebulosus was thought to possess a skin receptive to light from the following general observations: When representatives of this species were put in a large aquarium, with their eyes covered with black-kid, spectacle-shaped blinders, made concave by a few stitches on the circumference of each cover and held in place by a stitch through the skin on each side of the head, they swam for most of the time at the dark end of the aquarium. Thus they seemed to react negatively to light. Further, such fishes always started to swim on a change in the intensity of the general light in the laboratory. This reaction was used successfully in another line of experimentation in which it was desired to induce swimming activities.

A precise form of experiment to test the sensitiveness of the skin of these fishes to light was arranged in the following way: Although the blinders just described fitted closely over the eyes and it seemed improbable that light could reach the retina, it was though safest to experiment with fishes from which the eyeballs had been removed. The fishes thus operated upon remained surprisingly normal. They were alert, retained their spontaneity and fed well. It was interesting to note the effect of the operation on the chromatophores in the integument. The fishes became very dark colored—almost black—and remained so during several weeks of observation. Two blinded fishes (it was found impracticable to work with a single one) were placed in a round china dish suspended by wire springs from a wall bracket. This

device eliminated to a large extent disturbing vibrations set up by movements in the laboratory. Such protection was necessary, as Amiurus was found to be extremely sensitive to mechanical disturbances. The dish was surrounded by a double curtain of black cambric, and the laboratory darkened. There was only sufficient light within the curtain to enable one to see the outlines of the fishes faintly and to follow any movements they might make.

The light stimulus used was from an electric pocket flashlight of half a candle-power, placed in an aperture in the surrounding curtain and held 16 cm. above the level of the shallow water in which the fishes were.

Observations were made in two sets; the first a check set, the second final. As a check on the apparatus, set A was carried out as follows: a slight mechanical jar was given to the dish sufficient to start the fishes swimming. After they had come to rest again they were watched for a period of five minutes, and their spontaneous swimming activities recorded. At the end of the fifth minute the flashlight was placed in the aperture and held there unlighted for one minute. These observations constituted set A. In set B the fishes were again disturbed by a slight jarring of the dish, and when they had come to rest were again observed for five minutes. At the end of the fifth minute the flashlight was put in position and lighted. Care was taken that the act of lighting in itself did not cause a mechanical disturbance; it consisted of only a slight pressure on the side of the flashlight handle, which was held in the air and did not touch the apparatus containing the fishes. These observations constituted set B. The results of forty trials of this type of experiment, using four fishes, are given in table 1.

TABLE 1

Responses of blinded catfishes to light

	SET A	SET B
Spontaneous swimming movements in 40 tests of 5 minutes each	In very dim light: 7 movements	In very dim light: 5 movements
Swimming movements in 40 tests of not over 1 minute each	In very dim light: 1 slight movement	In bright light: In 1st 10 seconds 29 movements In 2nd 10 seconds 9 movements In 3rd 10 seconds 2 movements

The swimming movements during the one minute in bright light were quite unlike the spontaneous swimming movements in dim light in that they were accompanied by certain preliminary reactions. After the light had been turned on the fishes showed first, an increased respiratory rate, then a twitching of the barblets and fins and finally they swam about.

Fishes in which the skin had been anesthetized by applying to it a 20 per cent solution of magnesium sulphate for five minutes, gave no response to the bright light held over them for one minute. This condition persisted for half an hour. After this interval slight responses to light stimulation began to appear. Thus in thirty-two trials of one minute in the light, using three fishes, there were in the first ten seconds no swimming responses; in the second ten, two; in the third ten, five; in the fourth ten, five; in the fifth ten, six; in the sixth ten, three; while in the whole minute there were eleven failures to respond at all. It is thus evident that half an hour after anesthetization the skin of Amiurus begins slowly to regain its receptivity for light. The loss of skin sensitivity through magnesium sulphate does not necessarily inhibit responsiveness, for in other experiments it was found that fishes whose skin had been anesthetized by this means reacted well when the ear was stimulated. With the flashlight, however, no response was called forth during the first half hour. Only after this period, as the effect of the anesthetic wore off, did the responses to light slowly return.

From these observations I conclude that the skin of Amiurus, like that of some other fishes, contains receptive organs for light.

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THE EFFECTS OF ADRENIN ON THE DISTRIBUTION OF THE BLOOD

VI. Venous Discharge from the Thyroid Glands

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Evidence of sympathetic innervation of the thyroid gland has been brought forward by many observers. Rhinehart (1) has shown that anatomically the nerve supply to the thyroid comes from the cervical sympathetic and enters the gland along the course of the arteries. Sinakewitsch (2) in a physiological study of the innervation of the gland observed that sympathetic stimulation, after removal of the superior ganglion (cervical) or after paralysis of this ganglion with nicotine, produced no effect in the gland. He concludes from this that the sympathetic nerves pass by way of this ganglion to the gland. Briau (3), Sacerdotti (4), Sinakewitsch (2), Lübcke (5), Ossokin (6), Asher and Flack (7), Rahe and collaborators (8), Watts (9) and others all report vasoconstriction in the thyroid from stimulation of the cervical sympathetic nerves.

That adrenin stimulates the terminations of the sympathetic system is a fact no longer questioned. The effect of artificial stimulation of the sympathetic fibers to a given tissue should be duplicated, therefore, by the effects of intravenous administration of adrenin. In a recent search through the literature no direct evidence could be found as to the effect of adrenin on the circulation in the thyroid.

It has been shown in previous papers of the series (10), that adrenin can shift the circulation in favor of some tissues at the expense of others. In view of the fact that the adrenal secretion might influence the thyroid activity through its blood supply, it was considered that a careful study of the effect of adrenin on the circulation in the thyroid might give some pertinent evidence as to the interrelation of these two endocrine organs.

METHOD

The methods of investigation employed in this work have been those described in previous papers of the series (10). The majority of the determinations have been made on the venous outflow from the gland but a few plethysmographic observations have also been made. As before, dogs under ether anesthesia were used as experimental animals.

RESULTS

In the outflow determinations the inferior thyroid vein was usually cannulated. The blood pressure was recorded from the femoral artery as before described. The effects of adrenin ("adrenalin") in pressor, depressor and neutral injections and infusions were studied, the drug being injected into the femoral vein.

It was found, as might be expected from the recorded literature on the effect of stimulation of the sympathetics to the thyroid, that adrenin produces a diminution in the venous outflow from the gland. This was found to be true with all dosages. Figure 1 shows the results

Fig. 1. Showing effect of pressor infusion on venous outflow from thyroid gland. Dose 10 cc. 1: 200,000 "Adrenalin" in two minutes, from *a* to *b*. Dog weight 7 kilos.

obtained from a pressor infusion. The momentary increase in the outflow at the beginning of the pressor reaction is due probably to the blood in the vascular spaces being suddenly squeezed out by the contraction of the vessels. This momentary increase was also observed with pressor injections. That the constriction following the momentary increase in outflow is active is evidenced by the fact that the diminished outflow lasts throughout the pressor stage of the blood pressure reaction, both returning to normal at about the same time.

Quantitatively the constriction varied directly with the size of the dosage. With the larger doses it was found possible practically to stop the circulation in the gland until the greater part of the blood pressure reaction was completed.

Watts (9) has published a venous outflow graph from the thyroid showing a slight dilatation following the constrictor effect of electrical stimulation of the sympathetic nerves. In no case was this reaction evident from our adrenin injections. The trauma necessarily involved may have played a part in Watts' results. It was observed, however, as is shown in figure 1, that with the larger dosages, both injections and infusions, there was a slight tendency to over-recovery. This was found to be more marked toward the end of an experiment. With dosages near the threshold (fig. 2) the primary increase in outflow and

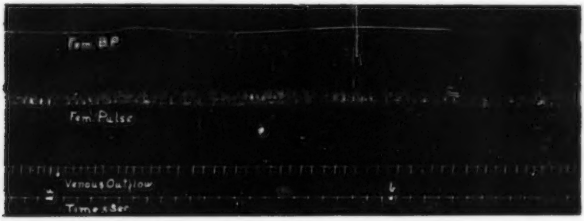

Fig. 2. Showing effect of a threshold infusion on venous outflow from thyroid gland. Dose 2.6 cc. 1: 200,000 "Adrenalin" in two minutes from *a* to *b*. Dog weight 7 kilos.

this over-recovery were not observed. This latter is probably to be explained as a temporary fatigue of the vessel musculature of the gland due to the extreme vasoconstriction preceding it.

As has been pointed out before, the physiological discharge from the adrenal glands is probably in minute quantities and extending over a variable period of time. In an investigation of this type, it is important that the experimental procedure simulate as nearly as possible the normal. Considerable attention therefore was directed to threshold dosages. The threshold of the reaction in the thyroid was found to be extremely low. Figure 2 shows the results of an infusion almost without effect on the blood pressure, which produced practically a 50 per cent reduction in the outflow from the gland. The apparent latent period in the graph is due to the time required for the passage of the adrenin solution through the venous cannula. The lowest threshold

observed was in a 20 kilo dog, the dosage being 0.2 cc. of a 1–1,500,000 dilution of adrenin or 0.000014 mgm. adrenin per kilo. The thyroid gland then, is the most sensitive in its reaction to adrenin of any organ so far studied and probably the most sensitive in the body.

Aside from the over-recovery mentioned above, in no case was dilatation observed to take place.

A few observations were made also on goiterous dogs, some of which presented the nervousness and rapid heart rate such as are seen in clinical exophthalmic goiter. In the glands of these dogs adrenin produced the same effect as in the normal glands.

In the few cases in which plethysmograph observations were made, adrenin in all dosages studied produced a diminution in the volume of the gland.

DISCUSSION

In the thyroid, intravenous injections of adrenin produce the same effects as those recorded from stimulation of the sympathetic nerves to the gland, namely, vasoconstriction. This would seem to point to an inhibition of the secretory activity of the gland.

Concomitant with an increase in the glandular activity there is usually a vasodilatation in the organ. This is a well recognized fact, for example, in case of the salivary glands. An agent producing an increased functioning of a tissue would be expected therefore, at the same time to produce a hyperemia of that tissue. An agent decreasing the functioning of a tissue would be expected at the same time to diminish the blood supply. Thus adrenin leads to inhibition of function in the kidney, accompanied by marked vasoconstriction.

The effect of sympathetic stimulation on the secretory activity of the thyroid gland has been investigated by a number of observers. Briau (3) asserts that the sympathetic nerves carry secretory and vasomotor fibers to the gland. Sacerdotti (4) believes the nerves to be without secretory function and to be vasomotor only. Katzenstein (11) observed marked degeneration in the thyroid following section of the sympathetic nerves and thus considers them to be secretory, a conclusion, however, that does not necessarily follow. Lübcke (5) considers the degenerative changes following section of the nerves to be due to a resulting change in the blood supply and thus the nerves to be without secretory function. Martini (12) after stimulation, and Horsley (13) some time after sectioning of the sympathetic nerves, could observe no histological changes in the glands and thus conclude that

the nerves are not secretory in function. Wiener (14) observed that there was no change in the thyroglobulin content of the gland after section of the vago sympathetic nerve and paralysis of the superior cervical ganglion. After resection of the inferior ganglion, however, with the superior intact, he observed a decrease in the thyroglobulin accompanied by a decrease in the weight of the gland. From these observations he concludes that the nerves are both secretory and trophic. Asher and Flack (7) postulated a secretion of the active substance of the thyroid into the blood stream following stimulation of the sympathetic nerves to the gland. They arrived at this conclusion from the observation that there was an increased excitability of the depressor nerve during or immediately after such stimulation. They state that intravenous injections of thyroid extract gave the same results. Rahe, Rogers, Fawcett and Beebe (8) observed a decrease in the iodine content of the gland following stimulation of the sympathetic nerves and conclude that the physiologically active substance of the gland is discharged into the circulation in response to nerve stimulus. Watts (9) working in Carlson's laboratory observed a decrease in the iodine and water content of the gland accompanied by vasoconstriction after electrical stimulation of the cervical sympathetic nerves. He observed also that mechanical interference with the blood flow from the gland produced the same results and concludes that all the changes produced by stimulation of the cervical sympathetic nerves can be explained by the resulting vascular changes. He further observed that no histological changes were evident following either procedure. Cannon and Cattell (15) have concluded that an increase of the action current in the salivary glands is a manifestation solely of the secretory activity. In the thyroid gland (16) they observed that sympathetic stimulation as well as injection of adrenin increased the action current and hence the secretory activity of the thyroid gland. They observed, however, no change in the action current due to occlusion of the arterial supply to the gland.

The weight of evidence in the literature seems to show that stimulation of the sympathetic nerves to the thyroid gland produces a diminution of the blood supply and at the same time an increase in the secretory activity of the gland. This is a surprising paradoxical phenomenon.

The accumulated metabolites in an anemic tissue may briefly stimulate as do other noxious substances. Such a stimulus, however, can hardly be considered physiologic in glandular tissue. The prolongation of a stimulus of this character would supposedly soon depress

the functioning. It is true that metabolitic stimulation is physiologic in nervous tissue as has been shown, for example, for the respiratory and vasomotor centers. Sollmann and Pilcher (17), however, have shown that in case of the vasomotor center, prolongation of metabolitic stimulation depresses and finally paralyzes the mechanism.

It would seem that the diminution in oxygen supply alone would result in an inhibition of function. It can safely be affirmed that anabolic activities, at least, are decreased by anemia. The thyroid gland, however, since it stores a considerable quantity of its active substance, may discharge periodically and in response to emergencies. If this be the case, no anabolic activities are necessarily in progress during the time of the discharge and the functional hyperemia which is observed in other tissues may not be an essential condition. This may explain the apparent paradox and vasoconstriction accompanied by an increase in the secretory activity of the thyroid may be physiologic.

SUMMARY

1. Adrenin in all dosages produces vasoconstriction in the thyroid gland.

2. There is a slight tendency to over-recovery in the outflow due supposedly to vascular fatigue.

3. The threshold is lower than in any other organ so far studied.

4. In no case was primary dilatation observed.

5. The literature seems to support the apparent paradox that an increased secretory activity of the thyroid is accompanied by vasoconstriction.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XLIII. Hunger and Appetite in Fever

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It has been observed that in conditions associated with fever there is usually an absence of hunger and appetite. This is true of the acute infectious diseases, of septic conditions—of the acute exanthematas. Osler (1) states that in typhoid fever there is as a rule a "complete loss of appetite, usually from the very onset—as in 825 cases, and almost invariably through the entire course." In acute and chronic cases of tuberculosis, appetite and hunger are either absent or diminished.

Carlson (2) noticed that tonsillitis, colds and antrum infections did not completely abolish the hunger contractions except when sufficiently severe to induce temperature elevations of 101° to 102°.

Dr. J. R. Rupp (3) found that when the temperature elevation reached 100° to 102° the strength and duration of the gastric hunger contractions showed practically no deviation from the normal. But they produced different effects on consciousness. Instead of the normal hunger pangs associated with increased appetite and desire for food, the gastric hunger contractions caused headache, nausea and epigastric disturbances like "sick stomach." More severe temperature reactions, 104° to 105°, usually rendered the stomach atonic and in that condition there are no hunger contractions. When the subject experienced chills there was a complete absence of hunger contractions.

Cannon (4) observed that when dogs were sick or febrile there was a lack of intestinal peristalsis. Carlson has observed that dogs with distemper or pneumonia refuse food, and show complete atony of the stomach and absence of the hunger contractions. The temperature of dogs affected with distemper ranges from 103° to 104°.

In the above conditions we are not dealing with any direct pathology of the stomach, intestines or peritoneum—for involvement of anyone of these per se or by its reflex action on the stomach, may cause of dis-

turbance of the hunger mechanism. We may justly assume that we are dealing with the effects of bacteria or toxins, which are either localized or circulating in the system.

It has already been emphasized that the predominant clinical feature in these conditions is the presence of fever. Fever has been defined as a reaction of the body against the invasion of bacteria or their proteins. It includes not only the elevation of temperature, which is the best clinical evidence of the disease, but also the associated changes in metabolism, the change in the urine, thirst, restlessness. Vaughn (5) believes that "protein fever" is identical with the condition of clinical fever. In view of the above facts we have undertaken the study of hunger and appetite in fever in order to determine:

1. The effect of fever on the hunger contractions.

2. If there is a depression of the gastric hunger contractions in fever to determine:

a. Whether this is due to mere temperature elevation;

b. Whether this is due to the bacterial toxins acting directly on the mechanism of the stomach;

c. Whether it is due to the action on the vagus or splanchnics—either by a lowering of vagus tone—or augmenting the inhibitory reflexes through the splanchnics.

I. THE EFFECT OF FEVER ON THE HUNGER CONTRACTIONS

Procedure

Simple gastric fistulas were made in a number of dogs in the usual manner. One week after the operation the experiments were commenced. The temperatures of all the dogs were then recorded for two days, on normal diet, at hourly intervals, and were found to vary from 100° to 101.8°. The dogs were then starved for twenty-four hours and the temperature recorded but showed no variation. After this control period the dogs were starved for twenty-four hours and then the hunger contractions were recorded. During periods of strong contractions the "pyrogenic substance" was injected either intravenously or subcutaneously. The injections themselves do not cause any interference with the hunger contractions. The temperature was recorded one-half to one hour after injection and at one-half to one hour intervals thereafter until the end of the experiment, at which time the dog was offered some appetizing food.

For the production of fever we used the following substances: sodium nucleate, a killed culture of bacillus prodigeosis, peptone, typhoid vaccine and caffeine citrate.

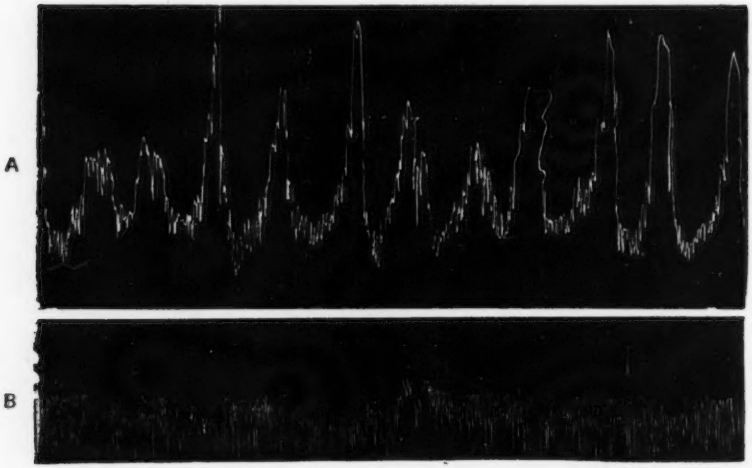

Fig. 1. *A*, tracing showing normal hunger contractions of a normal dog after twenty-four hours starvation. Temperature of dog 101°F. *B*, tracing showing gastric atony and absence of gastric hunger contractions of same dog after intravenous injection of sodium nucleate. At the time tracing *B* was taken the rectal temperature of the dog was 105.6°F.

Results

Intravenous injections of 10 cc. of 10 per cent sodium nucleate

Dog 1. The temperature rose from 100° to 105.8°F. The injection caused vomiting and immediate and prolonged depression of the hunger contractions. Observation period, 3½ hours. Dog refused food but drank water greedily. The temperature was normal on the next morning.

Dog 2. The temperature rose from 100° to 105.4°F. There was vomiting and prolonged depression of the hunger contractions. Observation period, 3½ hours. Dog refused food at end of period. Temperature normal in the morning.

Dog 3. The temperature rose from 100° to 105.8°F. There was immediate vomiting and prolonged depression of the hunger contractions. The dog refused food at the end of the period. Period of observation, 3 hours. Temperature normal in the morning.

Dog 4. The temperature rose from 101.8° to 102.8°F. There was prolonged depression of the hunger contractions. The period of observation was 2 hours, 35 minutes. Dog refused food; temperature normal in the morning.

Subcutaneous injections of 10 per cent Na nucleate solution

Dog 1. Temperature rose from 100.6° to 103.6°F. There is a gradual and prolonged depression of the hunger contractions. The hunger contractions not typical. There was no vomiting at any time. Observation period, 6 hours.

Dog 2. Temperature rose from 100.8° to 104°F. There were no hunger contractions present during the period of observation. Observation period, 4 hours. Dog refused food. Normal temperature in the morning.

Dog 3. The temperature rose from 100.8° to 105.2°F. There were no hunger contractions present; no vomiting at any time. Period of observation, 3 hours, 50 minutes. Animal refused food. Normal temperature next morning.

Dog 4. Temperature rose from 101° to 103°F. The immediate depression of the hunger contractions is marked. There was no vomiting at any time. Animal refused food—drank water only. Temperature was normal next morning.

Dog 5. Temperature rose from 100.8° to 103°F. The hunger contractions persisted through the period. The dog ate a small quantity (about 50 gms. of meat) at end of period. Observation period, 5 hours.

Dog 6. Temperature rose from 100.8° to 102.4°F. Hunger contractions were present.

Intravenous injections of killed culture of B. prodigiosis

Dog 1A. The injection was made at 10 a.m. Temperature of dog rose from 100° at 10 a.m. to 105°F. at 3 p.m. There was a complete cessation of the hunger contractions. The dog was extremely restless and at the end of the period drank water greedily Period of observation, 5 hours. Food refused at end of period. Temperature was normal the next morning.

Dog 1B. The temperature rose from 100.8° to 104°F. There was an absence of hunger contractions during the entire period. Period of observation, 2 hours, 25 minutes. Dog refused food at end of period. Temperature normal next morning.

Dog 2. The temperature rose from 100.6° to 103°F. The immediate effect was depression of the hunger contractions. Vomiting occurred once. Food refused. Temperature normal the next morning.

Dog 3. The temperature rose from 101.4° at 9.30 to 104.8°F at 12.45 and then fell to 103.6°F. at 1.37 p.m. No hunger contractions were present at any time during the febrile period. Dog refused food at the end of period. Period of observation, 4 hours. Temperature normal next morning.

Dog 4. The temperature rose from 101° at 9.30 to 104.2°F. at 12.45, and at 4 p.m. the temperature was 101.4°F. During the period from 10.30 a.m. to 12.45 p.m. when the temperature ranged from 103 to 104.2° there were no hunger contractions. At 3 p.m. with a temperature of 102.4° and later at 4 p.m. with temperature at 101.4° the contractions were present but irregular. Dog refused food. Period, 6 hours. Temperature normal next morning.

Effects of peptone (Witte's). Ten cubic centimeters of 5 per cent peptone—intravenous

Dog 1A. In this first experiment we injected 3 cc. of a 4 per cent solution. The temperature rose from 101.2° to 102°F. There was no effect on the hunger contractions.

Dog 1B. Ten cubic centimeters of 5 per cent Witte's peptone intravenous. Temperature rose from 101.4° at 10.53 to 102°F. at 1.10, and at 3.15 the temperature was 100.4°F. The immediate effect was vomiting, urination, defecation, dyspnea. The hunger contractions were temporarily inhibited but returned. Period of observation, 4 hours.

Dog 2. The temperature was practically unaffected by the injection, temperature at the outset being 100.8° and at the end of period was 101°F. The immediate effect was vomiting, urination, defecation, dyspnea, restlessness. The hunger contractions were temporarily abolished but returned, irregular in character. Period, 3 hours.

Dog 3. The temperature rose from 100.8° to 101.6°F. The immediate effects were vomiting, defecation, dyspnea. The hunger contractions were temporarily abolished, but there was later a return of normal contractions. Period of observation, 2 hours.

Dog 4. The temperature rose from 101.8° to 104.4°F. The immediate effect was a cessation of the hunger contractions, vomiting, defecation, urination dyspnea; respiration markedly increased. Period, 3 hours.

Subcutaneous injections of 10 cc. of 5 per cent Witte's peptone

Dog 1. There was no elevation of temperature. The hunger contractions were normal. Period, 3 hours.

Dog 2. The temperature rose from 101.4° to 102°F. The hunger contractions were present, although not regular. Vomiting occurred. Period, 3½ hours.

Effects of 0.7 gm. of caffeine citrate, intravenously

Dog 1. Temperature was not elevated. The hunger contractions were normal. Period, 3 hours.

Effects of intravenous injections of typhoid vaccine—500 millions

Dog 1. There was no elevation of temperature. The hunger contractions were normal. Period, 3 hours.

Dog 2. Temperature rose from 101.4°F. to 102°F. Normal hunger contractions were present throughout. Period, 4 hours.

Dog 3. Temperature rose from 101° to 101.8°F. Hunger contractions were present throughout period. Period, 4 hours.

SUMMARY

In general the results may be briefly summarized by the statement that on intravenous and subcutaneous injections of sodium nucleate there is a *depression of the hunger contractions.* The temperature is elevated as high as 105.8°F. (fig. 1) These dogs refuse food but drink water readily. There is vomiting and defecation after intravenous injection but not after the subcutaneous injections.

Killed culture of B. prodigiosis, when injected intravenously, will cause a temperature elevation of 103 to 105°F. and result in absolute cessation of hunger contractions. Food was always refused after the experiment but the next morning temperature was normal and dogs ate the food that was offered on previous evening.

Peptone. With the exception of one dog in which the injection of 0.5 grams peptone solution caused an elevation temperature to 104.6°, we were not able to cause any marked temperature elevation. In the dog 4—typical symptoms of anaphylactic shock were evident, although this was the first time the animal had been used. The hunger contractions were inhibited.

Typhoid vaccine. This proved a disappointment as the intravenous injection of 100 million to 500 million failed to produce a rise of temperature. There was no effect on the hunger contractions.

Caffeine citrate. Seven-tenths gram was injected intravenously but failed to cause any temperature elevation above 0.6° to 1°, which was not enough to influence hunger.

One dog developed a large fluctuating abscess. This dog had not been injected at any time either subcutaneously or intravenously. Temperature of dog was 103°. The hunger contractions of this dog were atypical and stomach tonus lowered.

From the foregoing experiments we feel justified in concluding that there is an absence of the hunger contractions in marked fever, that associated with this there is always the absence of desire for food and the animal will not eat as long as marked fever is present. It may be urged that the vomiting after intravenous injections is a factor in the depression of the gastric motor mechanism, but the depression is marked even when there is no vomiting, as in the subcutaneous injections.

II. THE MECHANISM IN THE DEPRESSION OF THE HUNGER CONTRACTIONS IN FEVER

The rôle of heat or temperature elevation

In endeavoring to determine the mechanism involved in the depression of hunger and gastric activity in fever the body temperature itself must be first considered, apart from any toxins.

To accomplish this external heat was considered the best means. This in itself, however, has many disadvantages for it is well known that dogs can easily regulate their body temperature through the respiratory system and keep the body temperature fairly constant.

It was hoped, however, that long exposures would serve to disable the heat regulating apparatus and possibly result in a condition of heat exhaustion, or possibly to a condition simulating heat stroke in man.

The following procedure was carried out: A simple wooden box, large enough to hold the dog comfortably was constructed and fitted with incandescent carbon electric lamps so protected that the dog

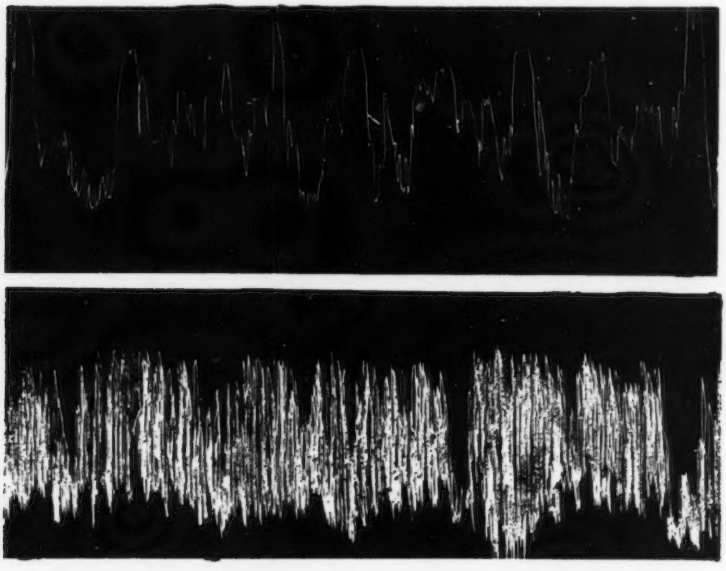

Fig. 2. *A*, tracing showing gastric hunger contractions of normal dog, rectal temperature 100°F. *B*, tracing showing absence of gastric hunger contractions of same dog after being confined in a room at the temperature of 39°C. for two hours. Temperature of dog when tracing *B* was taken, 100.5°F.

could not come in direct contact with them. The dog was placed in the box, the current was turned on and the hunger contractions were recorded in the usual manner by means of the rubber balloon. The animals, however, when so exposed became extremely restless, so that we found it impossible to record contractions while the animal was in the box. We therefore were obliged to expose the animal for one and three-quarters to two hours in the box, at a temperature of 35° to 37° and at the end of that period contractions were recorded. Two dogs were used—three experiments on dog 1, and one experiment on dog 2.

The results are summarized in the following:

Dog 1. The length of exposure was 1¼ hours. Temperature elevation negligible (101.8 to 102°). Temperature of box was 37°C. There were no hunger contractions.

2. Length of exposure, 1¾ hours. Temperature elevation negligible (101.8 to 102°). Temperature of box 37°C. Hunger contractions were normal.

3. Length of exposure, 2 hours. Temperature fell from 101.6° to 101°F. Temperature of box 37°C. Hunger contractions were present; later vomiting and a loss of stomach tone.

Dog 2. Length of exposure, 1 hour; temperature of box 37°C. Temperature rose from 101.4 to 103.6°F. in first hour, then fell to 102°F. in second hour, and to 100°F. in the third hour. There were no hunger contractions.

In general the results indicate that heat itself is a factor in the depression of gastric hunger mechanism. Just how it may do so is difficult to determine; but the following may be suggested: (a) Vascular effect—a general vasodilation—with an accompanying dilatation of the blood vessels of the stomach; or (b) that with increased heat of body there is an increase in CO_2 of blood which in turn might lead to a loss in gastric tone; (c) increased excitability of the inhibitory nervous mechanism; (d) lowered vagi tonus.

That external heat itself has a depressing effect on gastric hunger contractions is especially important with regard to gastric motility. It may be that the gastric derangements in young infants with the onset of warm weather may be due in part to the heat factor itself. It is conceded, of course, that the gastric enzymes and the food play a part in the disturbance.

Do the bacterial toxins have a direct depressor action on the stomach?

Methods. In order to test this the contraction of small strips of the stomach musculature were recorded according to the technique of Alvarez. We used mainly pyloric strips from the greater curvature, which we found after a number of trials to give best results. These strips were from stomach of freshly killed dogs. We did not find it necessary to use $BaCl_2$ to initiate contractions in any case. As toxins we used sodium nucleate, B. paratyphoid toxin (Ecker), tetanus and diphtheria toxins. To control our results we used adrenalin, pituitary extract, atropin and pilocarpin.

Results. The results of a series of ten experiments show that these toxins do not have any direct action on the stomach musculature proper, as determined on isolated strips (fig. 3).

Sodium nucleate failed to have any effect. B. paratyphoid toxin (prepared by Mr. Ecker) seemed to have a slight depressant action,

but this was true of the *boiled* as well as *unboiled* toxin and therefore may have been due to the salts used in its preparation. Tetanus and diphtheria toxin in extremely large doses seem to have little or no effect. We wish to note here that adrenalin causes a decrease in tone of the contractions of pyloric strips, that atropin causes a cessation and loss of tone of the contractions of pyloric strips and that pilocarpin causes an increased tone and increase in the individual contractions. This confirms the findings of Luckhardt, Tumpowsky and others on the action of these drugs on the hunger contractions.

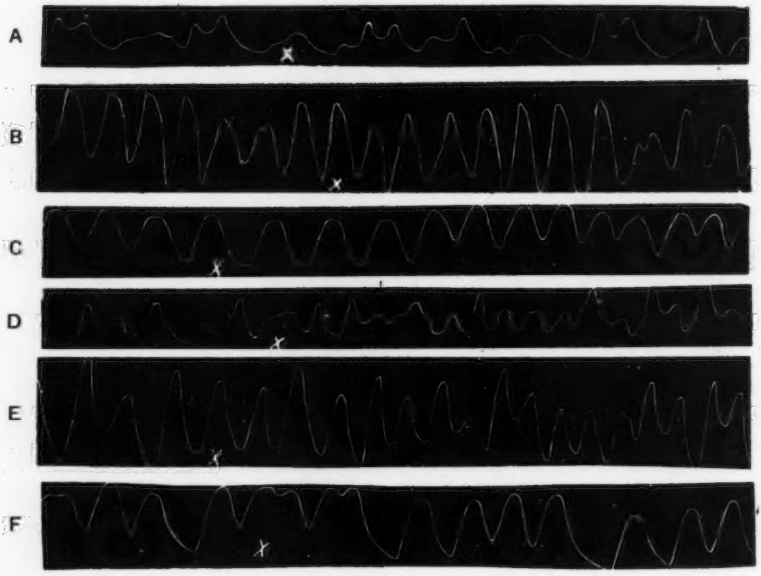

Fig. 3. Tracings from strips of the stomach muscularis (antrum region) bathed in oxygenated Ringer solution. At *X* the various toxins and sodium nucleate added to the Ringer solution. *A*, application of 1 cc. 10 per cent sodium nucleate to muscle strip. *B*, application of 3 cc. 10 per cent sodium nucleate to muscle strip. *C*, application of 1 cc. diphtheria toxin. *D*, application of 2 cc. diphtheria toxin. *E*, Application of tetanus toxin to muscle strip. *F*, Application of paratyphoid toxin to muscle strip. Showing no direct or immediate depressor action of these toxins on the gastric muro-muscular mechanism. The portion of the tracings reproduced represents a period of twenty minutes.

The rôle of the splanchnics and vagi

Rôle of splanchnics. The possibility that the fever and its toxins may cause inhibition of the gastric hunger contractions by way of the splanchnics must be considered. A dog was therefore prepared with both splanchnic nerves sectioned and the procedure was carried out as in part I.

The results on one dog show that hunger contractions are absent in the presence of fever even when the splanchnic nerves are cut.

Thus, summarizing the results of one experiment, the temperature rose from 101.8° to 105.6°F. The effect was immediate and prolonged cessation of hunger contractions and atony of stomach. Dog refused food. Temperature was normal in the morning.

Rôle of vagi. Do the bacterial toxins or the fever cause a lowering of vagus tone and therefore cause an absence of hunger contractions?

Both vagi were sectioned in each of three dogs, and a period of three months was allowed before any work was commenced. Autopsy confirmed operative procedure, as vagi were found cut in each of the dogs. The following is a summary of the observations:

Intravenous, sodium nucleate—10 cc. of 10 per cent in dogs with both vagi sectioned

Dog 1. The temperature rose from 101.6° to 106°F. The immediate effect was a forceful contraction of the abdomen but no vomiting. The stomach tonus was present and hunger contractions were present even at temperature of 105.8°. Period, 4 hours.

Dog 2. Temperature rose from 101° to 103°F. The hunger contractions were present throughout. Period, 2 hours.

Dog 3. Temperature rose from 102° to 103.2°F. Tonus and hunger contractions present.

Dog 3. Temperature rose from 101.4 to 103°F. Tonus present as well as hunger contractions.

Section of both splanchnics or both vagi

The results on one dog in general correspond to those obtained on section of the vagi. Temperature elevation was not obtained higher than 102.8°F. Hunger contractions were normal.

III. "INANITION FEVER"

In connection with the problem of hunger and appetite in fever, we became interested in the "inanition fever" of infants, so called, because it emphasizes the very close connection between the rise in temperature and the condition of inanition or starvation (Holt).

Essentially it is a condition occurring in the first five days of life in which there is an elevation of temperature which may vary from 102–104–106°F., *apparently due to the fact that the infant gets very little or nothing at the breast.* It is further characteristic that the temperature falls, when the child is put to the breast, or when artificial feeding, or even when water is given. (Holt.)

What might be the nature of the hunger contractions in such a condition?

We attempted to simulate this condition in two litters of pups.

Results

Litter 1. Two days old. Rectal temperature recorded every hour for two days after taken from the mother and thus prevented from nursing. The body temperature fluctuated from 101°F. to 94°F. with a tendency to a gradual decline towards the low figure. At no time did the body temperature of the starving pups rise above the normal.

Litter 2. One day old. Rectal temperature recorded every hour the second day while the pups were permitted to nurse. The body temperature fluctuated between 97°F. and 102°F., with an average of 100.5°F. The pups were then taken from the mother and their body temperature recorded hourly for three days. The temperatures fluctuated between 94°F. and 101.8°F., the average temperature during the starvation period being distinctly subnormal.

As the starvation did not induce fever, the hunger contractions were not recorded.

SUMMARY

1. There is an absence of hunger contractions and a lowering of the stomach tonus during fever in dogs when the temperature reaches 103°F. and above; this being especially true in the temporary fevers.

2. The temperature elevation itself is a factor in the depression of the stomach tonus and an inhibition of hunger contractions.

3. The splanchnic nerves do not appear to be involved in the inhibition. The fever or the toxins appear to induce a lowering of vagus tone, and this leads to a cessation of the hunger contractions. But we do not believe that this will account for absence of hunger during a fever lasting ten to twenty days.

4. Bacterial toxins, namely, B. paratyphoid toxin, B. prodigiosis, tetanus and diphtheria toxin, sodium nucleate, have no direct action on the contractions of stomach musculature, particularly of pyloric strips.

5. Starvation does not induce fever in newly born pups.

We wish to thank Dr. I. H. Tumpowsky and Mr. Seymour Cohen for assistance in part of this work.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XLIII. The Influence of Experimental Ulcers of the Stomach and Duodenum on the Hunger Contractions of the Empty Stomach

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The recent work of Ginsburg, Tumpowski and Hamburger (1) furnishes clear experimental evidence in support of the view that the so-called gastric ulcer pains or "hunger pains" of gastric and duodenal ulcers in man are due, at least in part, to the ordinary hunger contractions of the empty or nearly empty stomach. The excessive painfulness of the gastric hunger contractions in gastric and duodenal ulcer patients may be due to (a) excessive strength and duration of the gastric tonus and contractions, or to (b) an excessive excitability of the sensory nerves in the stomach wall. The tracings obtained from the empty or nearly empty stomach of ulcer patients at the time when ulcer pains are felt do not reveal contractions appreciably stronger than those of the empty stomach of the average normal persons. But since there are considerable individual variations in the gastric hunger contractions, observations on ulcer patients alone will not settle the question as we have no control records from these patients before development of the ulcers. Records of the gastric hunger contractions may be obtained on a patient during active ulcer and after healing of the ulcer, but the actual healing of the ulcer is difficult to establish without laparotomy, and after healing scar tissue formation and pyloric obstruction frequently complicate the gastric motility.

In the hope of obtaining conclusive data on this question the following work was undertaken with experimental ulcers of the stomach and duodenum on dogs, at the suggestion of Professor Carlson.

A series of three experiments is also reported showing the influence of gastroenterostomy on the gastric hunger contractions. Gastroenterostomy operations are still being made in ulcer cases with the idea

that this measure diminishes pain and accelerates the healing processes by diminishing the mehcanical and chemical irritation of the ulcer by the gastric content. The value of this operation in ulcers, except in cases of actual pyloric obstruction, has not been established, but it is possible that the altered chemical and mechanical conditions in the stomach following the gastroenterostomy operation may tend to depress the hunger contractions and thus diminish the ulcer pains.

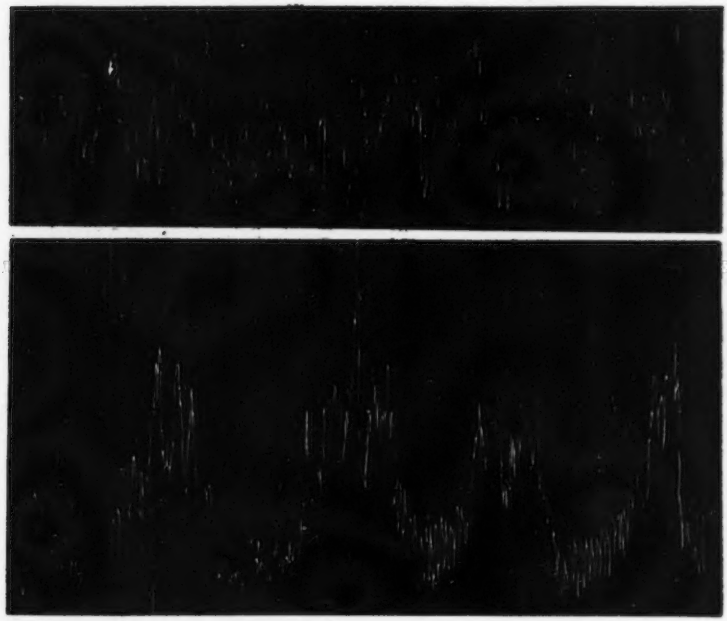

Fig. 1. Tracings showing typical hunger contractions of the empty stomach of the dog; *A*, before and *B*, five days after production of an ulcer in the antrum (lesser curvature). In the case of tracing *B*, the dog whined or moaned at the height of each group of hunger tetanus.

EXPERIMENTAL PROCEDURE

The work was carried out on dogs. The animals were kept under observation for varying periods prior to the laparotomy and ulcer production for the purpose of establishing the normal gastric hunger

contractions. Gastric hunger contractions were recorded by the balloon method as described by Carlson (2). The balloon and connection tube were inserted by way of the mouth. The dogs were trained to lie without restraint on the table near the recording apparatus and all but one became accustomed to sleep during the experiments. The observation period began twenty-four to thirty-six hours after the previous feeding, so as to assure a stomach free from food.

The normal hunger contractions vary considerably within certain limits with regard to frequency and amplitude, so that the average type can only be gained by a large series.

The ulcers were produced by silver nitrate injections into the submucosa, as described by Dragstedt (3).

Short loop posterior gastroenterostomies were performed. The anastomosis was placed on the antrum of the stomach. Pylorectomy was performed by dividing the pylorus and closing the cut ends. Upon recovery from the operations the motility of the empty stomach was again recorded for the purpose of comparison with the normal.

Results with duodenal and gastric ulcers

Dog 1. The normal peristalsis of the empty stomach of this dog was recorded for seventeen days. At the end of this time a silver nitrate ulcer was produced on the anterior wall of the duodenum one inch from the pylorus. For fifteen days following the production of the ulcer the gastric hunger contractions were recorded. Autopsy at this time revealed the presence of a partially healed ulcer 1.5 cm. in diameter at the site of the silver nitrate injection.

There was no definite increase in the gastric hunger contractions following the duodenal ulcer in this case.

Dog 2. The normal hunger contractions of this dog were recorded for nineteen days. At the end of this time the silver nitrate ulcer was produced in the stomach on the lesser curvature one-half inch from the pylorus. Upon recovery from the laparotomy the hunger contractions were recorded for eleven days.

Results. After the production of the ulcer the gastric hunger contractions were slightly greater in amplitude; though less in frequency than the normal. At autopsy forty days after the silver nitrate injection, it was found that the ulcer had healed.

Dog 3. Normal hunger contractions were recorded for three days. An ulcer was then produced in the anterior duodenal wall one inch from the pylorus. After the operation the gastric hunger contractions were recorded for eleven days.

Results. A comparison of the tracings before and after producing the ulcer revealed an increase over the normal amplitude of about 60 per cent.

Dog 4. Normal gastric hunger contractions were recorded for three days. An ulcer was then produced on the lesser curvature of the stomach one-half inch from the pylorus. After the operation gastric motility was recorded for twenty-six days.

Results. After the production of the ulcer the gastric hunger contractions were more frequent than the normal. At autopsy twenty-seven days after the operation it was found that the ulcer had healed.

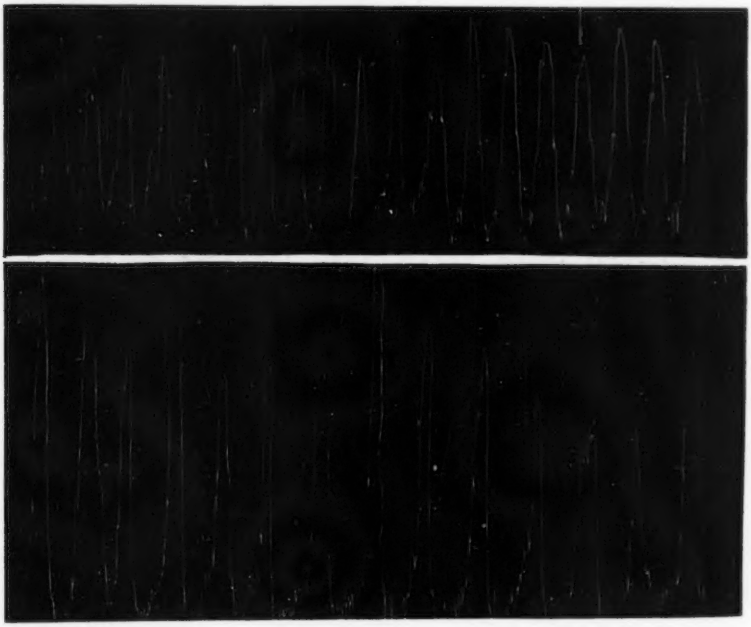

Fig. 2. Tracings showing typical hunger contractions of the empty stomach of the dog; *A*, before, and *B*, four days after production of an ulcer in the duodenum one inch below the pylorus.

Results with gastroenterostomy

Dog 5. Normal gastric hunger contractions were recorded for six days. Posterior gastroenterostomy was then performed. After the operation the gastric hunger contractions were recorded for fifty-five days.

Results. On the first two days (April 16, 17) fair motility was present. Then for nine days the movements of the empty stomach were absent. During the rest of the observation period there were days of inactivity intervening with days of moderate motility and only on two days did the hunger contractions approach the normal in amplitude and vigor.

Dog 6. An ulcer was produced on the lesser curvature near the pylorus. After recording the gastric motility for eleven days, a gastroenterostomy was performed The gastric hunger contractions were registered for eight days after the operation.

Results. The motility of the stomach after the gastroenterostomy was not noticeably different from that preceding the operation.

Dog 7. The gastric hunger contractions were recorded for three days. Gastroenterostomy and pylorectomy were then performed. The hunger contractions of the stomach were recorded for eight days after the operation.

Results. The contractions of the empty stomach were not appreciably affected by the operation.

SUMMARY

In three out of four dogs with ulcers in the duodenum or the pyloric end of the stomach the motility of the empty stomach was greater than normal. But this augmentation of the gastric hunger contractions was not sufficiently marked to account for the excessively painful character of these contractions in ulcer patients. It is therefore probable that the hunger pains in gastric and duodenal ulcers in man are not due to excessive hunger contractions, but to hyperexcitability of the sensory nerves in the stomach wall, as suggested by Carlson.

The effects of gastroenterostomy on the gastric hunger contractions are variable, with a tendency to depression. The hypomotility, when present, may be due to valve-like action of the intestine opposite the stoma causing retention of the food in the duodenum and hence a reflex inhibition of the stomach from the duodenum (4) as well as regurgitation through the stoma.

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ON THE GENESIS AND INHIBITION OF EXTENSOR RIGIDITY

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INTRODUCTION

Despite the extensive physiological studies on "decerebrate rigidity" ((9), (10), (11), (12), etc.) little is yet known of the exact centres involved in its genesis and inhibition.[1] It is a phenomenon of extensor rigidity in which the tonic activity of nerve and muscle (12) holds the unconscious organism in the posture of reflex standing. Hence its relation to problems of locomotion and muscle tone is important.

In this investigation we wished to gather data bearing especially on the inhibitory mechanism and its effect on the character of the extensor rigidity. Accordingly, decerebrated cats were so suspended that levers, attached to their tonically contracting extensor muscles, would record on a drum the tone changes of these muscles. The cortex of the anterior lobe of the cerebellum was stimulated electrically and inhibition of the rigidity was recorded, preponderantly in the ipsilateral muscles. This had been previously reported by other investigators (15) (9) (13). Next the cortex was removed and the electrode placed deeply in the subcortical structures; on stimulation here more marked inhibition was obtained and it was more strictly ipsilateral. Finally the anterior lobe of the cerebellum was removed, exposing the dentate-rubral tracts, which on stimulation gave still more marked and more purely ipsilateral inhibition. Furthermore it was found that by subsequent ablation of the mid-brain with its red nuclei, the inhibitory effects of stimulating the cerebellar structures were abolished; it also was noted that the remaining extensor tonus (referable to the hind-brain) differed from the characteristic decerebrate rigidity of the mid-brain preparation.

[1] Throughout this paper the word "genesis" is used as meaning "the main reflex center," the ultimate source of the rigidity being the proprioceptive receptors.

METHOD OF INVESTIGATION

Normal cats were used in all these experiments. They were etherized, the trachea was cannulated and the carotid arteries were ligated to control bleeding. Next the animal was turned over and through a median, fronto-occipital incision, the parietal bones were laid bare so that the chisel decerebration, on Sherrington's decerebrating apparatus (6) could be quickly performed. We found this method more satisfactory than the old operation through a trephine hole for several reasons; in the first place much time is saved, so the animal has little ether; secondly, the whole anterior part of the calvarium is quickly split off, making easy removal of the fore-brain and giving free access to the mid-brain; and thirdly, if subsequent transections are desired, the free exposure allows accurate choice of the point to be cut. During the decerebration and subsequent removal of the fore-brain the vertebral arteries were tightly held by an assistant; in this way no blood was lost and no clots formed around the medulla or cerebellum. Occasionally, however, this hemostasis was imperfect and the resulting clots impaired or ruined the preparation. As soon as all brain tissues above the transections had been removed, the anterior and middle fossae of the cranial cavity were washed out with warm saline solution and then packed with dry cotton, care being taken that no pressure was exerted on the brain-stem. Only after all this had been done was the pressure removed from the vertebral arteries. Clotting had usually taken place on the surface of the cut brain-stem, and no further trouble from hemorrhage occured unless the animal had asphyxial convulsions, or other severe activities that raised the blood pressure. When such emergencies arose, the packing had to be soaked off with hot saline solution (about 40°C.) and the vertebrals again held so that clots might reform.[2]

When this operation was finished the cat was usually in a state of good rigidity and was placed in a box warmed with electric lights to about 34°C. Here the animal was supported on a shelf through which holes had been cut for the legs. All four legs, therefore, were thrust downward through these holes and had free play, the support under the thorax and abdomen holding the animal up. In the bottom of the box were holes through which wires from the leg muscles could pass to the recording levers without letting in much cool air. The front of the box was of glass so the preparation could be continually observed,

[2] For instruction in most of this technique I am indebted to Dr. Alexander Forbes.

and the window at the cranial end, covered with a curtain, allowed access to the cat's head for operating on the brain-stem and for stimulating. The recording apparatus was a standard Harvard kymograph, at low speed, and the durations of the stimulating induction shocks were recorded by a signal magnet in series with the primary coil.

To record the variations in rigidity caused by various stimulations, extensor muscles prominently involved in the tonic reflex were attached to the levers. The front limbs were most frequently used; the triceps of each foreleg after being freed from its attachment at the elbow, was connected with the recording lever by copper wire; a 60 gram weight was hung on each lever directly below the attachment of the wire. These wires passed from muscle to lever by way of a small hole in the bottom of the box. When the hind limbs were used (as was done in a few experiments) the Achilles tendons were freed and, in the same way, attached by wires to their levers. The legs were held firmly in clamps so that only the recording muscle registered its changes in tonus. It was also found necessary to hold the head fixed in a vice because asymmetrical stimuli would cause unilateral contractions or relaxations of the neck muscles, and if movement of the head was permitted these changes were transmitted to the shoulder girdle and displaced the forelegs, thus falsifying the record of the triceps contractions.

When the animal was safely in the thermostat and seemed to be in satisfactory condition, the packing in the skull was soaked in warm saline and removed; the tentorium was then rongeured away and the desired portions of the cerebellum exposed. When the superior peduncles were to be stimulated, they were exposed by separating the posterior colliculi from the anterior lobe of the cerebellum with blunt dissection; a prism-shaped piece of this anterior lobe was then cut away and the rest of the cerebellum elevated and pushed back with a spatula. If done carefully, with the vertebral arteries held, and if plenty of cotton pledgets soaked in warm saline were used for sponging, the superior peduncles came plainly into view, and might be easily reached with a stigmatic electrode.

Induced tetanizing currents were used for stimulating; they were applied usually through unipolar electrodes, but in some cases through bipolar. The coil used was not standardized in any units; estimates of the strength of stimulus were made by placing the electrodes on the experimenter's tongue, and recording the coil distances for threshold and maximal sensory stimulation.

At the end of each experiment the brains were fixed in 10 per cent

formalin and later the parts of interest were cut in serial sections. In this way blocks above and below each transection could be studied; the exact position of each cut was found, and the presence or absence in the preparation of such important structures as the red nuclei was definitely determined in relation to the concomitant physiological observations. For the preparation of these many hundreds of microscopical sections we wish here to thank Miss Bisson and Miss Lyons, who skilfully and quickly carried out this laborious part of the investigation.

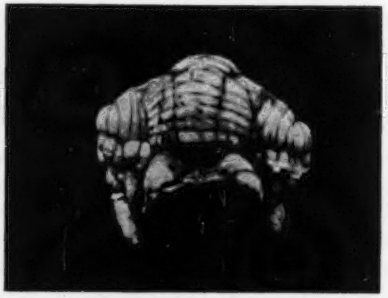

Fig. 1. Anterior view of brain-stem of a cat, showing transected mid-brain and points *L*, *M* and *R* where stimuli were applied to the cortex of the anterior lobe of the cerebellum.

BRIEF PROTOCOLS OF EXPERIMENTS

Cat 9. October 4, 1916

9.20 Decerebration on Sherrington decerebrator.
9.30 Put animal in thermostat. Weak[1] rigidity developing.
10.00 Increasing rigidity, greater on right than left.
10.30 Strong[1] rigidity in all four legs.
10.55 Apparatus adjusted; levers attached to gastrocnemii.
11.20 Strong rigidity; tentorium removed and lobus anterior of cerebellum exposed.
11.50 Strong rigidity; stimuli applied to cerebellar cortex with unipolar electrode. Area *L* (see fig. 1) gives ipsilateral contraction of gastrocnemii, with strong currents painful to tongue. Cat retains rigidity until killed.

Autopsy. Shows transection to have been above the third nerves. Serial sections of brain-stem show lower half of red nucleus present and intact.

[1] For definitions of these adjectives as applied to rigidity, see table 2.

Cat 10. October 21, 1916

9.20 Decerebration. Weak rigidity immediately.
9.40 Less rigidity.
10.05 Dissection of triceps for attachment to levers.

Fig. 2. Inhibition of decerebrate rigidity by stimulation of cerebellar cortex. Upper tracing is right triceps, lower is left triceps. Time in five second periods. At *R-7*, the cortex of the anterior lobe of the cerebellum is stimulated to the right of the mid-line (see *R*, fig. 1) with resulting ipsilateral inhibition and contralateral slight contraction. At *L-7* the cortex is stimulated on the left (see *L*, fig. 1) with slight bilateral inhibition. At *L-6* the coil distance is decreased 1 cm. and the cortex again stimulated on the left, with marked bilateral effect. (See protocol of cat 10.)

10.25 Strong rigidity.
10.45. Lobus anterior of cerebellum exposed. Greatly increased rigidity.
11.30. Strong bipolar stimuli (painful on tongue) applied to cerebellar cortex at *L*, *R* or *M* (see fig. 1) cause bilateral inhibition of the rigidity, in some cases slightly more marked ipsilaterally (see fig. 2). Records taken until 1.20; rigidity variable during this time. Increased at will by stimulating Gasserian ganglion, saphenous nerve, anus or vulva.
3.30. Cat still in good condition; killed.

Autopsy. Shows transection between superior and inferior colliculi, slanting forwards. Serial sections show the lower large cells of the red nucleus to be present, but invaded with hemorrhage.

Cat 12. October 20, 1916

2.55. Decerebrated.
3.10. Lobus anterior of cerebellum exposed. Tricipes dissected out. Weak rigidity.
3.42. In thermostat; apparatus applied. Rigidity increasing.
4.05. Unipolar stimulation to cerebellar cortex. No inhibition. Rigidity
to variable; easily elicited by passive movements or asphyxia.
4.10.
4.45. Extreme rigidity; lasting four minutes (probably due to asphyxia.) Animal killed.

Autopsy. Shows transection to have passed through interpeduncular ganglion and anterior end of pons. No red nucleus seen in serial sections.

Cat 16. November 11, 1916

1.10. Decerebration with spatula.
1.30. Onset of rigidity. Tricipes prepared. Cat in thermostat.
1.32. Clonus develops in right triceps quickly followed by left.
2.12. Lobus anterior of cerebellum exposed. Stimuli applied with bipolar electrodes, at *R* and *L*. Inhibition obtained only by stimulation at *R* and even these are not well marked reactions.
2.45. Removed cortex of anterior lobe of cerebellum, a sliver about 3 mm. thick being taken off. Electrode thrust into subcortical tissue near right superior peduncle; stimuli here gave marked inhibition ipsilaterally and contralateral contraction (see fig. 3). Optimum strength was with coil at 7 cm. when electrode on tongue gave a slight tingling sensation.
3.30. Cat killed.

Autopsy. Serial sections of brain-stem show lower two-thirds of red nucleus present and apparently intact.

Cat 17. November 1, 1916

9.35. Decerebration and immediate exposure of anterior lobe of cerebellum. Part of anterior lobe cut away to expose the superior cerebellar arms.
9.50. Tricipes prepared for attachment to levers.

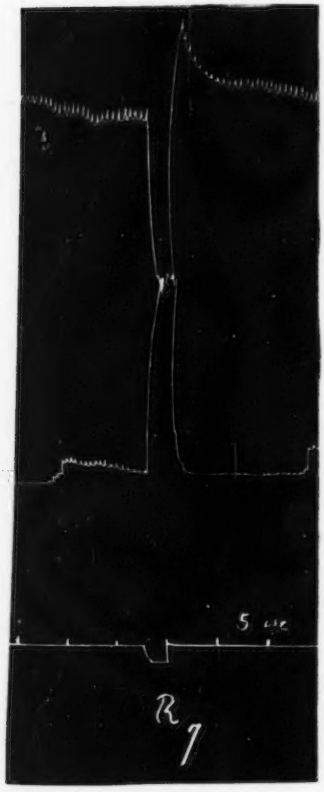

Fig. 3. Inhibition of decerebrate rigidity by cutting away cortex of right anterior cerebellar lobe and thrusting electrodes into subcortical tissue near the right superior peduncle. Result: A marked ipsilateral inhibition with contralateral contraction. Upper tracing—right triceps muscle. Lower tracing—left triceps muscle. (See protocol of cat 16.)

10.00. In the thermostat; levers attached and stimuli begun with unipolar electrode. Stimulation of the peduncles (with current just felt on the tongue) gives inhibition sometimes, but not strictly ipsilateral. A source of error however, lies in the free movement of the cat's head which changes the position of the shoulders. Similar weak stimuli to the right red nucleus region of the cut mid-brain give ipsilateral inhibition. Slightly stronger stimuli applied to the mesial fraction (fronto-pontine

bundle?) of the left crus cerebri cause distinct ipsilateral inhibition of the tonic extensor contraction, with slight contralateral inhibition. This was repeated six times in succession; the left superior peduncle (dentate-rubral tract from left dentate nucleus to right red nucleus) was then cut, and the inhibitory effect could no longer be elicited by stimuli as previously applied.

Autopsy. Transection passes through posterior edge of superior colliculi, slants forward, and leaves the third nerves uninjured. No serial sections made but it is evident from the gross examination that at least the lower part of the red nucleus was left in the preparation.

Cat 18. November 25, 1916

10.45. Decerebration, weak rigidity develops quickly.
11.00. Anterior lobe of cerebellum exposed and partly removed to expose superior peduncles. Strong rigidity; increasing.
12.15. Levers attached to tricipes; head immobilized in vice.
12.20. Stimuli applied with unipolar electrode to right and left superior peduncles.
to (These are of such strength as to give a distinct tingling sensation to
3.00. the tongue.) Ipsilateral inhibition of the tonic contraction of the triceps with contralateral increase of contraction, results regularly (see fig. 4). Stronger stimuli give contraction on both sides. Weaker stimuli occasionally give inhibition on both sides. Stimulation of middle peduncles gives contraction on both sides. Stimulation of mesial fraction of left crus cerebri (fronto-pontine bundle?) gives ipsilateral inhibition of the rigidity regularly, usually with slight contralateral inhibition also (these stimuli are just perceptible to the tongue). The middle peduncles were sectioned one after the other, and each section caused a distinct increase in rigidity.
3.00. Cat killed.

Autopsy. Serial sections of mid-brain show lower half of red nucleus present and intact.

Cat 19. December 2, 1916

9.15. Decerebration with exposure and removal of the anterior lobe of the cerebellum to expose superior peduncles.
9.45. Rigidity strong; cat in thermostat; levers attached to tricipes.
11.00. Rigidity still increasing; stimuli to peduncles occasionally give ipsilateral inhibition with contralateral contraction, occasionally inhibition bilaterally. Often no effect, or with strong stimuli bilateral contraction.
12.45. Cat giving irregular responses; rigidity becoming variable.
2.04. Cut first right and then left superior peduncle; former has no effect, latter causes bilateral increase of rigidity lasting two minutes.
2.10. Cat dies in convulsions with great rigidity.

Autopsy. Serial sections of brain-stem show about 1200 micra of red nucleus remaining below transection. The left superior peduncle is cut cleanly; the right is cut, but along with it both the other right peduncle and surrounding structures are injured.

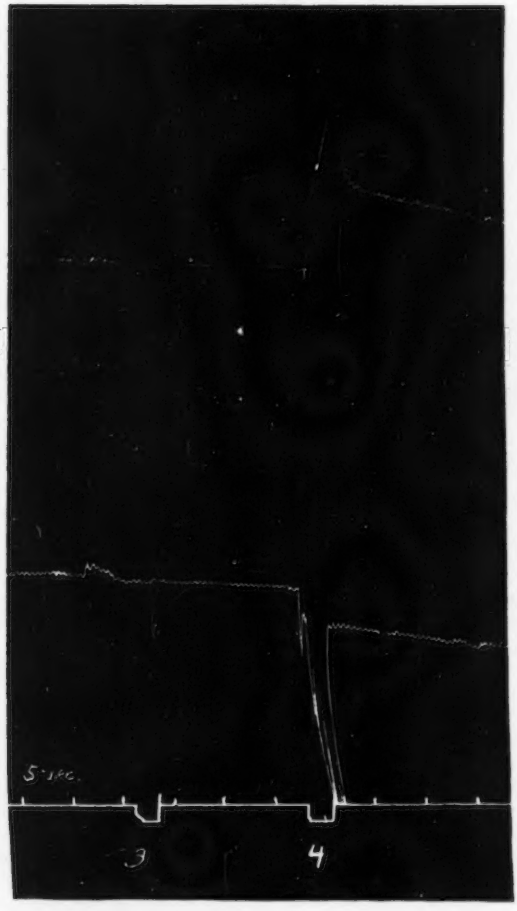

Fig. 4. Inhibition of decerebrate rigidity by stimulation of left superior peduncle. Result: Ipsilateral inhibition with contralateral contraction of triceps muscles. Upper tracing—right triceps. Lower tracing—left triceps. (See protocol of cat 18.)

Cat 23. January 13, 1917

Preparation A

9.40. Decerebration with immediate weak rigidity.
9.50. Removal of tentorium and anterior lobe of cerebellum.
10.00. In the thermostat; strong rigidity.
10.50. Progressive movements with hind legs; front legs clamped. Stimuli to superior peduncle cause inhibition of the contralateral tonic contraction of the triceps, with increase ipsilaterally. Quick contractions and relaxations, however, continue to be superimposed on the tonic variations.

Preparation B

10.55. Mid-brain again transected and block of tissue removed. Progressive movements stop. Stimuli applied to superior peduncles now have no effect on tonic contraction of triceps. Rigidity extreme.
11.45. Rigidity still extreme, tail raised, legs stiff.
1.15. Rigidity still extreme, tail raised, legs stiff.
2.15. Rigidity still extreme, tail raised, legs stiff. Cat killed.

Autopsy. Serial sections show that preparation *A* contained about 3400 micra of red nucleus, while preparation *B* is just below this nucleus and contains a little inferior colliculus and the pons but no red nucleus.

Cat 24. January 20, 1917

Preparation A

9.30. Decerebration. Strong rigidity comes on immediately, steady. Anterior lobe of cerebellum exposed.

Preparation B

10.07. Second transection; through anterior mid-brain. Rigidity remains strong.

Preparation C

10.30. Third transection; through posterior mid-brain. Great increase in rigidity; tail raised.
10.37. Strong rigidity.
11.15. Great spasm with extreme rigidity. Cerebellar cortex stimulated with unipolar electrode; no inhibition.
11.40. Strong rigidity. Anterior lobe of cerebellum removed exposing superior peduncles. Stimulation of peduncles gives no inhibition of rigidity.
12.25. Rigidity still strong. After trying many areas in the hind-brain, inhibition was twice obtained bilaterally, from unipolar stimulation of the floor of of the ventricle in the region of the vestibular nuclei. This was an inhibition which lasted until other stimulations were given.

Preparation D

12.45. Fourth transection. Increase in rigidity during cutting, soon passes off.
12.50. Weak rigidity.
12.57. Strong rigidity.
1.10. Better rigidity than at 12.57.
1.25. Respiration labored; rigidity variable, gone one minute and present the next.
1.40. Convulsion with extreme extensor rigidity and death.

Autopsy

Preparation A. Serial sections show geniculate bodies and upper reticular part of red nucleus.

Preparation B. Sections contain the whole of the large-celled part of the red nucleus, extending for 2,200 micra antero-posteriorly.

Preparation C. Upper sections show inferior colliculi and tip of pons; lower one show decussation of fourth nerve.

Preparation D. Sections show the fifth nucleus and the upper part of the pons.

Cat 30. February 13, 1917

Preparation A

10.15. Decerebration. Immediate strong rigidity.
10.30. Exposed cerebellar cortex, including lobus anterior, lobulus simplex and crus primum (14). Rigidity increases. Animal put in thermostat.
11.40. to 12.14. Rigidity strong and fairly constant. Levers attached to tricipes and bipolar stimuli applied to different points on the cerbellar cortex. With strong stimuli (painful to tongue, coil distance 8 cm.) inhibition is obtainable at points *L* and *R* (see fig. 1) but effect is not ipsilateral regularly, it is sometimes bilateral and occasionally contralateral. With the electrodes thrust 4 mm. into the cortex, strong stimuli (8 cm. to 15 cm. coil distance) give variable effects. With weak stimuli (just perceptible on tongue, 18 cm. coil distance) regular ipsilateral inhibition is obtained at *L* and *R*, the electrode being thrust 4 mm. into the cortex.

Preparation B

12.15. After getting the above results four times in succession from stimuli at *L*, the mid-brain was again quickly transected, to cut out the red nuclei. The rigidity immediately increased, and stimuli (repeated just as above) at *L* gave no inhibition. Other points tried, but no inhibition obtainable.
12.40. Rigidity still strong.
12.42. Breathing becoming labored.
12.55. Cat dead.

Autopsy

Preparation A. Decerebration transection through superior colliculi, red nuclei and crura cerebri; about 1600 micra of the lower part of the red nucleus is left in on each side, but is invaded with hemorrhage.

Preparation B. Second transection: through inferior colliculi and upper tip of pons. No red nucleus.

Cat 24. February 24, 1917

9.40. Decerebrated, immediately weak rigidity.
9.45. Rigidity strong; exposed anterior lobe of cerebellum.
10.05. Levers attached to tricipes; animal in the thermostat.
10.40. Rigidity still fair; began stimulation of cortex; bipolar.
10.50. Rigidity extreme. No inhibition with bipolar stimuli; with unipolar: inhibition ipsilaterally, and contralateral contraction, from stimuli (too weak to feel on tongue) applied on cortex below point *R* (see fig. 1). With electrode deep in cortex at point *M* (fig. 1) obtain bilateral inhibition.
11.25. Excised anterior lobe of cerebellum and exposed superior peduncles; seemed to have injured the peduncles. Rigidity weak.
11.40. No inhibition obtainable from stimuli to peduncles. Rigidity becomes strong when animal is handled.
12.25. Variable rigidity; becomes strong if limbs are passively moved.
12.45. Variable rigidity; becomes extreme if limbs are passively moved. Cat killed.

Autopsy. Transection is anterior to superior colliculi, so red nuclei are present. Serial sections show injury to right superior peduncle, none to left.

Cat 34. February 27, 1917

9.40. Decerebration through thalamus; slight rigidity.
10.05. Cat in thermostat; begins progression movements.
10.55. Cut brain-stem back to superior colliculi; progression stops; legs flaccid for two minutes, and then rigidity comes on.
11.10. Strong rigidity. Operation on tricipes.
11.40. Weak rigidity; increased by passive motion.
12.20. Weak rigidity, steady.
1.40. Weak rigidity, steady.
3.00. Exposed anterior lobe of cerebellum; rigidity increased. Levers attached to tricipes.
3.10. Bipolar stimuli to *R* and *L* (fig. 1) surface of cortex give ipsilateral inhibition, sometimes contralateral, sometimes bilateral.
4.20. Cat killed.

Autopsy. The first transection was above the posterior commissure; the second was through the upper part of the red nucleus, leaving in 1400 micra of the small celled reticular part, and all of the large celled posterior part.

EXPERIMENTAL RESULTS

A. On inhibition. By correlating the data in the above protocols it is found that ten preparations responded with inhibition of the extensor tonus to stimuli applied to the anterior cerebellar lobe or to the superior cerebellar arm. Three preparations gave no such response. It was then shown by the histological studies that the ten cases giving inhibition were those in which the dentate-rubral path was intact, with at least part of the red nucleus present; while the three in which no inhibition could be elicited had had the red nucleus removed. Furthermore, in two of these cats (30 and 23) after demonstrating the presence of the intact inhibitory mechanism (preparations 30A and 23A)

TABLE 1

CAT NO.	RED NUCLEUS (PRESENT OR ABSENT)	RESULT OF STIMULATING ANTERIOR CEREBELLAR CORTEX OR SUPERIOR CEREBELLAR PEDUNCLE
23A	Present	Inhibition
30A	Present	Inhibition
32	Present	Inhibition
16	Present	Inhibition
9	Present	Inhibition
34	Present	Inhibition
17	Present	Inhibition
1	Present	Inhibition
19	Present	Inhibition
10	Present	Inhibition
12	Absent	No inhibition
23B	Absent	No inhibition
30B	Absent	No inhibition

a second transection, further back, was shown to abolish inhibition (see table 1). Subsequent anatomical studies of the serial sections revealed that the main difference between these preparations (those with and those without inhibition) lay in the absence of the red nuclei in the latter, or at least in the cutting of the dentate-rubral path. In fact in cat 17, where the red nuclei are left in, the inhibitory function is present before, and absent after the cutting.

In one case (24C) inhibition of the rigidity was elicited after the removal of the red nuclei, but it was brought on not by stimulation of the cerebellar cortex or dentate-rubral path, but of the floor of the ventricle near the vestibular nuclei. Besides, this inhibition was not typical of that elicited by stimulation of the peduncles—which gives a

quick fall in muscular tone returning to its former level immediately on discontinuing the stimulus (figs. 2, 3, 4) but an inhibition with slower and more lasting relaxation of rigidity.

Gradation of the strength of stimulus was an important factor. If too strong currents were used nothing but clonic contractions of the registering muscles were obtained (cats 1 and 19); by reducing the strength a point could be found where inhibition resulted. But this point was far from constant under the best conditions. When areas on the cortex of the anterior lobe of the cerebellum were stimulated (*L* and *R*, fig. 1) stronger currents were necessary to bring out inhibition than when the deeper structures, or the peduncles themselves, were stimulated (cat 30). In fact very weak stimuli (not perceptible or hardly perceptible to the tongue) when applied to the superior peduncle, produced this inhibition, whereas strong stimuli (painful to the tongue) had to be used on the cerebellar cortex.

It is also interesting to note in this connection that the cortical stimuli are diffuse in their effects. Such stimulations usually but not always, give bilateral inhibition (9) (15), stronger on the ipsilateral side than contralaterally (cat 10), but when the electrodes are thrust into the cortex (cat 30) the effect is more definitely ipsilateral, and is most definite of all when the superior peduncle is exposed and stimulated. These superior peduncle stimulations frequently gave pure ipsilateral inhibition with no effect contralaterally, or with contralateral contraction (cats 9, 16, etc., fig. 4).

These findings can be reconciled in an interesting way with various observations of Sherrington (9), Weed (15) and Brown (3) on the inhibition of decerebrate rigidity. But before entering upon the more detailed discussion it will be well to state briefly the current explanation of the phenomena.

Decerebrate rigidity is a tonic contraction of those extensor muscles of the body which tend to hold the animal in a typical standing position; in Sherrington's words it is a "postural" reflex—a "stell-reflex," according to Magnus (4). This rigidity only takes place when the cerebrum and thalamus have been removed; so it is supposed that the inhibitory influences which normally check this tonic extension are exerted on some "tonus centres," in the mid-brain or hind-brain, by the cerebrum (3) (10). These centres normally give rise to extensor tonus, but are controlled from above by inhibitory influences and cause rigidity only when that control is removed. If such is the case there must be some definite inhibitory pathway, and this has been shown by Weed

(15) to lie in the "anterior mesial" part of the internal capsule and in the "mesial fraction" of the crus cerebri; perhaps this is, then, the fronto-pontine bundle or bundle of Arnold. Stimulation of this area on the cut surface of the mid-brain caused ipsilateral inhibition of the existing rigidity, an observation which we repeated on two of our cats (17 and 18). Moreover Weed traced the pathway into the contralateral middle cerebellar peduncle, showing that destruction of this middle pontile arm stopped the inhibitory action of stimuli applied to the above mentioned point in the crus. Further than this, he found what he considered the possible cortical representation of this tract in the superior vermis. Stimulation of this cortex caused inhibition of the rigidity, and extirpation of it caused a marked increase in rigidity. As we have shown above, our experiments corroborate the inhibitory effect of stimulating this part of the cerebellar cortex. But we think that cortical stimulation merely causes inhibition by spread to the dentate-rubral path, because of the following facts: (a) that we have not always obtained an increase of rigidity ipsilaterally by extirpation of the anterior part of the lobus anterior; (b) that we have found subcortical stimulation more effective than cortical, and (c) that we have found direct stimulation of the underlying superior peduncle most effective of all, and most definitely ipsilateral in effect. There is in all probability a cortical link in the inhibitory path, but we have not been able to satisfy ourselves as to its location.

From these observations, it seems fair to tentatively propose a pathway for the inhibitory influences which control decerebrate rigidity. Weed has traced this through the internal capsule and crus (probably as fronto-pontile bundle) to the pons, thence up the opposite middle cerebellar arm, and possibly to the cortex of the lobus anterior. We present evidence that this pathway leaves the cerebellum in the superior cerebellar arm (brachium conjunctivum). The course of these fibers in the cerebellum is not known. Anatomical works give ambiguous statements as to the termination of axones arising from pontile cells (7). All we can state is that the inhibitory path that ascends to the cerebellum in one brachium pontis, leaves the cerebellum by the brachium conjunctivum arising from the contralateral nucleus dentatus. A stimulus, therefore, to the brachium conjunctivum which arises from the right dentate nucleus, will inhibit the tonic activity of the left red nucleus. This, in turn, inhibits the rigidity of the right side of the animal, because of the decussation of the rubro-spinal tract.

If we accept this as the inhibitory path, the theory that the genesis

of the extensor tonus lies in the red nuclei becomes most plausible. Weed (15) and Brown (2), (3) have shown that stimulation of the cut mid-brain in the region of these nuclei causes contralateral extension of the limbs. It is in the red nucleus that the inhibitory path seems to end. Therefore we may reason that normally the red nuclei give rise to tonic impulses causing an extensor posture, that these are controlled from above by inhibition along the pathway described, and that cutting this pathway allows over-activity of the nuclei and hence decerebrate rigidity.

B. On genesis. In order to determine more exactly the relation of the red nuclei to decerebrate rigidity, other experiments were carried out. In these, cats were prepared as above described and their brain-stems were transected at levels both above, below and through the red nuclei. Notes on the duration and character of the resulting phenomena were made, and these were compared with the anatomical findings in serial sections of the brain-stems. In some cases observations of the effect of transecting at different levels were made on the same cat, as, for example, in cat 23, which was transected three times. The first was with the Sherrington decerebrator and was far forward in the posterior part of the thalamus. After this the animal had marked rigidity for an hour and then began to show progressive movements of the legs. As this interfered with the rigidity, a second cut was made further back, stopping the progressive movements and throwing the animal into steady rigidity for an hour and a quarter. Subsequent microscopical examinations of this block of brain-stem showed that the transection passed through the posterior commissure and mammillary bodies, leaving the whole of the red nucleus intact. The third transection was made through the inferior colliculi and anterior edge of the pons, thus eliminating the red nucleus; but rigidity remained for three and a half hours. This will serve as an example of the procedure, and in this way eighteen preparations were examined but in most cats only one transection was made and the phenomena observed were recorded and compared with those of other cats cut on different levels. The data collected are correlated in table 2.

By examining this table it is seen that the first nine preparations contained intact red nuclei which were presumably functioning, while in the last nine the red nuclei were either absent or severely injured and therefore not functioning. Taking these two groups and comparing their physiological activities, it is seen that in the first nine all but two had steady rigidity, and all but two had strong rigidity, while in

TABLE 2

	CAT NO.	THE LEVEL OF TRANSECTION IS THROUGH:	RED NUCLEUS	CHARACTER AND DURATION OF RIGIDITY..... EXTREME STRONG WEAK NONE STEADY OR VARIABLE
1	23A	Posterior commissure and mammillary bodies	Present	Strong, at times extreme, for 1½ hrs.
2	32	Geniculates and mammillary bodies	Present	Strong at end of 3 hrs., variable
3	16	Anterior reticular part of red nucleus, above large cells	Present	Weak, with clonus for 1½ hrs.
4	9	Geniculates and anterior end of red nucleus	Present	Weak rigidity for 2½ hrs.
5	24A and B	A. Mammillary body and bundle of Vicq D'Azyr. B. Anterior small celled part of red nucleus	Present	Strong for 1 hr., steady.
6	34	Anterior small celled part of red nucleus	Present	Strong at end of 4 hrs., following decline from 1 hr. to 3½ hrs.
7	11	Anterior edge of large celled part of red nucleus	Present	Variable for 5 hrs., extreme at end.
8	1	Large celled part of red nucleus and III nucleus	Present	Strong for 1½ hrs.
9	19	Large celled part of red nucleus	Present	Strong for 2½ hrs., then weak and variable for 2½ hrs. more
10	10	Between superior and inferior colliculi and posterior end of large celled part of red nucleus	Present Injured	Weak variable rigidity for 6 hrs.
11	20	Inferior colliculi and anterior edge of pons	Absent	Weak rigidity for 1 hr., increased by passive motion induced for ¾ hr. more
12	25	Inferior colliculi and anterior edge of pons	Absent	Weak rigidity for 3 hrs., with variations to strong and extreme both spontaneously and with passive motion.
13	12	Anterior tip of pons	Absent	Weak and variable for 1½ hrs., extreme could be induced
14	23B	Inferior colliculi and anterior pons	Absent	Strong rigidity for 3½ hrs.
15	24C	Inferior colliculi and anterior pons	Absent	Varies from strong to extreme for 2½ hrs.
16	21	IV nucleus and pons	Absent	Slow onset, weak and variable for 1 hr., then strong for 1½ hr.

TABLE 2—*Continued*

	CAT NO.	THE LEVEL OF TRANSECTION IS THROUGH:	RED NUCLEUS	CHARACTER AND DURATION OF RIGIDITY..... EXTREME STRONG WEAK NONE STEADY OR VARIABLE
17	14	Pons and medulla just anterior to V nucleus	Absent	Weak for 20 minutes
18	22	Trapezium and anterior part of Bechterew's nucleus	Absent	None

Definitions of terms used for describing rigidity in table 2

"Extreme" rigidity = Head and tail both elevated, legs all fully and strongly extended.

"Strong" rigidity = Slight stiffness of neck muscles, proximal third of tail arched, legs all stiffly extended.

"Weak" rigidity = Hind legs resist flexion, but can be flexed with pressure; forelegs stiff and fully extended. Neck and tail show slight, if any stiffness.

"None" or "No" rigidity = Position of flaccid flexion, i.e., legs can be easily flexed and extended.

the last group of nine, a weak, variable rigidity is the rule. The most interesting point, however, cannot be shown in the table. This is a difference in the character and spontaneity of the rigidity. In the first group, where the red nuclei are functioning, the rigidity comes on spontaneously and is fairly steady; this is the typical "decerebrate rigidity" described by Sherrington. In the second group, where the red nuclei are absent, there is a kind of extensor tonus but it is more variable and depends on afferent stimuli being frequently applied to the affected limbs. In other words, where the red nucleus is absent, rigidity appeared only when induced by massaging the muscles, moving the limbs passively, and by such procedures as rolling the animal over on its back. This extension lasts from two to ten minutes and must then be renewed by some similar procedure.

This is not always the case, as is seen in preparations 23B and 24C, but in dealing with such a complicated organism as a decerebrated cat there are many factors that cannot be controlled. In these two, for instance, there were previous transections which threw the animal into strong rigidity before the red nuclei were excised, this, perhaps, conditioning the subsequent reflexes. The amount of blood lost at operation is variable, also, and may account for such irregularities as are seen in preparations 6 and 9, which were weaker than the rest of that group.

There seems, therefore, to be enough general difference to make the grouping of interest and at least to suggest (a) that the red nucleus has a rôle in the maintenance of the steady spontaneous rigidity, and (b) that a kind of extensor tonus has its main reflex centre in the hind-brain, independent of the red nucleus.

When the transections go further back and reach the vicinity of the trapezium and the vestibular nuclei, even this tonic extension is lost. But these tonic extensor phenomena of the middle and posterior hind-brain segments should be investigated further, and on their own merits. Sherrington (10) (11) (9) and Thiele (13) have suggested that the vestibular nuclei might be of importance in decerebrate rigidity. Sherrington ruled out the possible influence of the semicircular canals by cutting the vestibular nerve at the internal auditory meatus and (9, p. 326) then producing the typical rigidity by decerebration. This does not prove, however, that afferent stimuli coming up the direct cerebellar tracts may not play upon the vestibular nuclei, either directly (8) or via the roof nuclei (1) and fastigio-vestibular tracts (5) and thus reflexly cause extensor tonus down through the vestibulospinal tract.

The difference in character between the typical decerebrate rigidity of the mid-brain preparation, and the variable tonic extension of the hind-brain preparation may be explained on the basis of the red nuclei. When the red nuclei are present there is a steady rigidity (if the operation be well performed). This rigidity can be inhibited by stimulating any point on the inhibitory pathway, and this path largely ends in the red nucleus. Therefore it seems that a large part of the motor activity arises in these nuclei. When cut further back so as to destroy this reflex (and incidentally the afferent fibers of Gowers' tract which go up the superior cerebellar arm) the tonic extension becomes less, but it can still be elicited by increasing the quantity of afferent stimuli. Even in cord lesions, some types of rigid extension appear and Magnus (4) in a recent paper (not yet received in this country) contrasts the postural reflexes of "pons animals" with those found in animals with the mid-brain intact. From the ontogenetic point of view, Weed's paper on *The Reactions of Kittens after Decerebration* (16) brings in more evidence to show that we are dealing with a complicated mechanism, the activities of which are graded according to the level of integration of the animal as well as the level of transection of the neural axis. It is hoped that further investigation will throw more light on a

subject, the practical application of which is so great in relation to such problems as clonus, contracture, muscle tonus and body posture.

In closing we wish to express our thanks to Dr. Adolf Meyer for his guiding interest in these experiments.

CONCLUSIONS

1. Stimulation of the superior cerebellar arm in decerebrate cats causes ipsilateral inhibition of their extensor rigidity.

2. The inhibition of decerebrate rigidity by stimulation of the cortex of the anterior lobe of the cerebellum (9) (13) (15) appears to depend on stimulation of the underlying superior cerebellar arm.

3. A kind of extensor rigidity (not the typical "decerebrate rigidity" of Sherrington) is found in preparations transected below the red nucleus. This extensor tonus is lost when the transection injures the vestibular nuclei.

4. Stimulation of the superior cerebellar arm elicits no inhibition of extensor tonus when the red nuclei are not left in the preparation or when the dentate-rubral path is cut.

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